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BIOLOGICAL THERAPY IN RHEUMATOID ARTHRITIS – EPIDEMIOLOGICAL STUDIES

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Biological Therapy in Rheumatoid Arthritis – Epidemiological Studies

THESIS FOR DOCTORAL DEGREE (Ph.D.)

by

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Defense of the thesis will take place on Wednesday 28th of
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Life is like a weird clinical study: we are not sure if it is controlled or observational; it is single-blind; the primary endpoint is poorly defined; the methodology is complicated with significant variability among individuals and countries; the mortality is 100%; and the principal investigator, if there is one, is surely laughing...

K.C.

Sub specie aeternitatis

B. Spinoza (1632-1677)

To my mother

ABSTRACT

The landscape of RA treatment has unquestionably changed dramatically during the last decade. A deeper understanding of the pathophysiological and immunological mechanisms in RA, earlier and more aggressive treatment, and the development and introduction to daily clinical practice of a new class of anti-rheumatic drugs, the so-called biologic therapies, has contributed to this 'revolution'. To date, nine biologic agents have been approved for the treatment for RA and more molecules with distinct mechanisms of action are currently being tested in laboratories and in clinical trials. In all cases, very good clinical efficacy and safety were documented in large, randomized, controlled clinical trials that led to regulatory approval.

However, not all questions regarding the optimal use of these agents can be addressed in randomized trials. Observational studies based on registries can provide important information about the effectiveness and safety of biologics in real-life RA populations as well as better insight of different treatment strategies. Thus they are important 'pieces of the puzzle' and can help complete the picture of RA treatment.

This thesis comprises of two parts: part I is based on four studies about several aspects of rituximab use in RA which are based on a large international cohort. The second part is based on four studies about the use of TNF inhibitors in RA (cycling, switching and discontinuation) which are based on local and national registers and a pilot clinical trial.

LIST OF SCIENTIFIC PAPERS

This thesis is based on eight original papers. They are listed below and will be referred to in Roman numerals.

- I. **Chatzidionysiou K**, Lie E, Nasonov E, Lukina G, Hetland ML, Tarp U, Gabay C, van Riel PL, Nordström DC, Gomez-Reino J, Pavelka K, Tomsic M, Kvien TK, van Vollenhoven RF.
Highest clinical effectiveness of rituximab in autoantibody-positive patients with rheumatoid arthritis and in those for whom no more than one previous TNF antagonists has failed: pooled data from 10 European registries.
Ann Rheum Dis. 2011 Sep;70(9):1575-80
- II. **Chatzidionysiou K**, Lie E, Nasonov E, Lukina G, Hetland ML, Tarp U, van Riel PL, Nordström DC, Gomez-Reino J, Pavelka K, Tomsic M, Kvien TK, van Vollenhoven RF, Gabay C.
Effectiveness of disease-modifying antirheumatic drug co-therapy with methotrexate and leflunomide in rituximab-treated rheumatoid arthritis patients: results of a 1-year follow-up study from the CERERRA collaboration.
Ann Rheum Dis. 2012 Mar;71(3):374-7.
- III. **Chatzidionysiou K**, Lie E, Nasonov E, Lukina G, Hetland ML, Tarp U, Ancuta I, Pavelka K, Nordström DC, Gabay C, Canhão H, Tomsic M, van Riel PL, Gomez-Reino J, Kvien TK, van Vollenhoven RF.
Effectiveness of two different doses of rituximab for the treatment of RA: data from the CERERRA collaboration.
Manuscript
- IV. **Chatzidionysiou K**, Lie E, Nasonov E, Lukina G, Hetland ML, Hauge E, Pavelka K, Gabay C, Nordström DC, Canhão H, Tomsic M, van Riel PL, Gomez-Reino J, Ancuta I, Kvien TK, van Vollenhoven RF.
Retreatment with rituximab in Rheumatoid Arthritis in a real-life cohort-data from the CERERRA collaboration.
Manuscript
- V. **Chatzidionysiou K**, Askling J, Eriksson J, Kristensen LE, van Vollenhoven R; for the ARTIS group.
Effectiveness of TNF inhibitor switch in RA: results from the national Swedish register.
Ann Rheum Dis. 2014 Jan 15 [Epub ahead of print]

- VI. **Chatzidionysiou K**, van Vollenhoven RF.
Rituximab versus anti-TNF in patients who previously failed one TNF inhibitor in an observational cohort.
Scand J Rheumatol. 2013;42(3):190-5.

- VII. **Chatzidionysiou K**, Askling J, Eriksson J, Kristensen LE, van Vollenhoven R.
Effectiveness and drug-survival of certolizumab pegol in clinical practice—results from a national registry
Manuscript

- √III. **Chatzidionysiou K**, Turesson C, Teleman A, Knight A, Lindqvist E, Larsson P, Cöster L, van Vollenhoven R, Heimbürger M.
A Multicenter, Randomized, Controlled, Open-Label Pilot Study of the Feasibility of Discontinuation of Adalimumab in Rheumatoid Arthritis Patients in Stable Clinical Remission.
Manuscript

Related publications

- I. **Chatzidionysiou K**, van Vollenhoven R.
When to initiate and discontinue biologic treatments for Rheumatoid Arthritis?
J Intern Med. 2011 Jun;269(6):614-25

- II. van Vollenhoven RF, Ernestam S, Geborek P, Petersson IF, Cöster L, Waltbrand E, Zickert A, Theander J, Thörner A, Hellström H, Teleman A, Dackhammar C, Akre F, Forslind K, Ljung L, Oding R, **Chatzidionysiou K**, Wörnert M, Bratt J.
Addition of infliximab compared with addition of sulfasalazine and hydroxychloroquine to methotrexate in patients with early rheumatoid arthritis (Swefot trial): 1-year results of a randomised trial.
Lancet. 2009 Aug 8;374(9688):459-66.

- III. van Vollenhoven RF, Geborek P, Forslind K, Albertsson K, Ernestam S, Petersson IF, **Chatzidionysiou K**, Bratt J; Swefot study group.
Conventional combination treatment versus biological treatment in methotrexate-refractory early rheumatoid arthritis: 2 year follow-up of the randomised, non-blinded, parallel-group Swefot trial.
Lancet. 2012 May 5;379(9827):1712-20.

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LIST OF ABBREVIATIONS

ACPA	Anti-citrullinated protein antibody
ACR	American College of Rheumatology
ADA	Adalimumab
Anti-CCP	Anti-cyclic citrullinated peptide
CDAI	Clinical Disease Activity Index
CRP	C reactive protein
DMARD	Disease Modifying Antirheumatic Drug
DAS	Disease Activity Score
ESR	Erythrocyte Sedimentation Rate
ETA	Etanercept
EULAR	European League Against Rheumatism
HAQ	Health Assessment Questionnaire
INF	Infliximab
MCP	Metacarpophalangeal (joint)
MTX	Methotrexate
PIP	Proximal interphalangeal (joint)
RA	Rheumatoid Arthritis
RCT	Randomized Clinical Trial
RF	Rheumatoid Factor
RTX	Rituximab
SDAI	Simplified Disease Activity Index
SJC	Swollen Joint Count

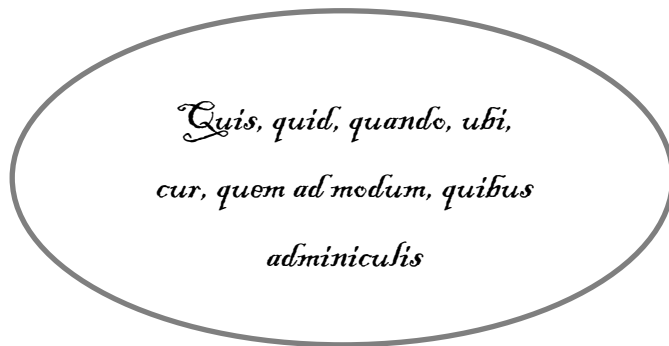
TJC	Tender Joint Count
TNFi	Tumor Necrosis Factor inhibitor
VAS	Visual Analogue Scale

1 INTRODUCTION

1.1 RHEUMATOID ARTHRITIS

Hermagoras (Ἑρμαγόρας, fl. 1st century BC), of Temnos, was an Ancient Greek rhetorician of the Rhodian school and a famous teacher of rhetoric in Rome. He introduced the method of dividing a topic under study into its ‘seven circumstances’: what, who, when, where, why, in what way, by what means (*Quis, quid, quando, ubi, cur, quem ad modum, quibus adminiculis*), which provided the roots of the ‘5W’s’ used widely today in several investigatory processes¹.

Here I will briefly describe the disease of interest of this thesis based on these ‘seven circumstances’.



1.1.1 ‘What’ – definition, classification criteria of RA

Rheumatoid Arthritis (RA) is a chronic, systemic, inflammatory disease of unknown etiology which is generally thought to be autoimmune in nature. It typically affects the small and medium joints and causes synovial inflammation which can lead to cartilage and bone destruction if untreated.

To classify RA different sets of criteria have been used over the last years. Until recently the 1987 revised ACR criteria were used² (table 1A), but the last 3 years new criteria have been proposed and accepted for clinical use (table 1B)³. The new criteria aim to earlier diagnosis, since they focus more on the presence of auto-antibodies (rheumatoid factor and anti-citrullinated peptide antibodies) and they do not include radiological changes. These classification criteria are mainly

used in research and as a support for clinicians, but they are not diagnostic criteria, and the clinicians should never forget that they cannot be used as such.

Criterion	Definition
1. Morning stiffness	Morning stiffness in and around the joints, lasting at least 1 hour before maximal improvement.
2. Arthritis of 3 or more joint areas	At least 3 joint areas simultaneously have had soft tissue swelling or fluid (not body overgrowth alone) observed by a physician. The 14 possible areas are right or left PIP, MCP, wrist, elbow, knee, ankle and MTP joints.
3. Arthritis of hand joints	At least 1 area swollen (as defined above) in a wrist, MCP or PIP joint
4. Symmetric arthritis	Simultaneous involvement of the same joint areas (as defined in 2) on both sides of the body (bilateral involvement of PIPs, MCPs or MTPs is acceptable without absolute symmetry).
5. Rheumatoid nodules	Subcutaneous nodules over bony prominences or extensor surfaces, or in juxtaarticular regions, observed by a physician
6. Serum rheumatoid factor	Demonstration of abnormal amounts of serum rheumatoid factor by any method for which the result has been positive in <5% of normal control subjects
7. Radiographic changes	Radiographic changes typical of rheumatoid arthritis on posteroanterior hand and wrist radiographs, which must include erosions or unequivocal bony decalcification localized in or most marked adjacent to the involved joints (osteoarthritic changes alone do not qualify)

Table 1A. The 1987 RA classification criteria. At least 4 of these 7 criteria had to be satisfied for classification of a patient as having RA. Criteria 1-4 had to be present for at least 6 weeks.

Criterion	Score
A. Joint involvement	
1. 1 large joint	0
2. 2-10 large joints	1
3. 1-3 small joints (with or without large joint involvement)	2
4. 4-10 small joints (with or without large joint involvement)	3
5. >10 joints (at least 1 small joint)	5
B. Serology	
1. Negative RF and negative ACPA	0
2. Low-positive RF or low-positive ACPA	2
3. High-positive RF or high-positive ACPA	3
C. Acute-phase reactants	
1. Normal CRP and normal ESR	0
2. Abnormal CRP or abnormal ESR	1
D. Duration of symptoms	
1. < 6 weeks	0
2. \geq 6 weeks	1
<p>Target population: patients who</p> <ol style="list-style-type: none"> 1. Have at least 1 joint with definite clinical synovitis 2. Patients symptoms and findings cannot be better explained by another disease 	

Table 1B. The 2010 ACR-EULAR classification criteria for RA. A score of $\geq 6/10$ is needed for classification of a patient as having definite RA.

Is RA an autoimmune disease? According to Witebsky a disease must fulfill three criteria to be considered autoimmune: 1) autoantibodies or a cell-mediated immune response against an autoantigen has to be present; 2) the respective autoantigen is known and 3) a similar disease can be imitated in animal models

based on an analogous immune response⁴. Based on these three requirements, the classification of RA as an autoimmune disease remains today somewhat controversial. Although several autoantibodies have been identified, the identity of a dominant arthritogenic autoantigen remains unclear. Moreover, while inflammatory arthritis can be induced in animal models, such as collagen induced arthritis, its direct relevance to human disease has been difficult to prove.

Two of the most well studied and described autoantibodies in RA are rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA). RF is an autoantibody against the Fc part of human IgG (figure 3). RF is most often an IgM molecule, but it can also be found as IgA or IgG. They can create immune complexes activating complement in the joint of RA patients which can in its turn increase vascular permeability, increase chemotactic factors and attract immune cells to the joint⁵. RF has a relatively low specificity for RA since it can be present in other systemic autoimmune diseases, for example systemic lupus erythematosus, but also in infections as well as in healthy, elderly individuals.

Anti-citrullinated protein antibodies are more recently discovered and in contrast to RF they have much higher specificity for RA, more than 95%⁶⁻⁸. Citrullination is a process by which arginine residues are modified (“deiminated”) to citrulline in the presence of high calcium concentrations by an enzyme called PAD (peptidyl arginine deiminase). It is a physiologic enzymatic process important for the degradation of intracellular proteins during apoptosis. ACPAs are important both for the initiation of disease but also for diagnosis⁹. They have also been shown to be associated with higher risk for erosive disease and thus poorer prognosis¹⁰. Several citrullinated antigens have been described in RA, with citrullinated fibrinogen, alpha-enolase, vimentin and collagen type II having been studied better. There is also some evidence that ACPA are involved in the etiopathogenesis of RA¹¹. ACPA can be measured by generic tests, which use synthetic peptides or mutated recombinant proteins. The test most commonly used is anti-CCP.

1.1.2 Who' and 'When' – epidemiology of RA

RA is a common disease with a prevalence of 0.5% - 1%. It is associated with considerable regional variation. Prevalence estimates for Southern European countries are lower than for Northern Europe, while highest rates are found in North America¹². In some Native American tribes up to 5% of individuals are affected, while in certain parts of rural Africa the disease is said to be absent. Women are predominantly affected in a ratio of 3:1. The mean age of patients at the time of diagnosis is the fifth decade. It has long been documented that RA clusters in families: the likelihood that a first-degree relative of a patient will share the diagnosis is 2–10 times the population prevalence of the disease, and recurrence risks are highest for relatives of the most severely affected index cases.

1.1.3 'Where' – clinical presentation of RA

The mode of onset, clinical presentation and course of the disease is highly variable. Some patients may have an acute onset with dramatic and systemic symptoms, such as fever, weight loss, polyarthritis and extra-articular manifestations, while other may have a more insidious onset, which is more common. RA affects predominantly the small to medium size joints of the body in a symmetrical manner. Inflammation in the joints leads to swelling, pain, limited movement and stiffness. Besides the joints, RA affects other organs as well, such as the lungs (interstitial lung disease, pulmonary nodules, pleural effusions), the heart (such as myocarditis and pericarditis) and mucosal glands (secondary Sjögren's syndrome)^{13,14}. Neurological and hematological manifestations are also seen. Rheumatoid nodules, both in juxtaarticular regions and in the lungs are the most common extra-articular manifestation of RA. Rheumatoid vasculitis is also an uncommon but well-described RA manifestation. RA increases the risk of cardiovascular events and certain malignancies, such as lymphoma¹⁵⁻¹⁷. The life span of RA patients is reduced by approximately 7 years. For these reasons RA should be considered a systemic disease (figure 1).

A characteristic feature of RA is its heterogeneity. Both in a genetic and clinical lever it can differ substantially among patients. Additionally the severity and prognosis can also vary significantly among individuals with RA. This heterogeneity makes the disease both challenging and interesting to investigate.

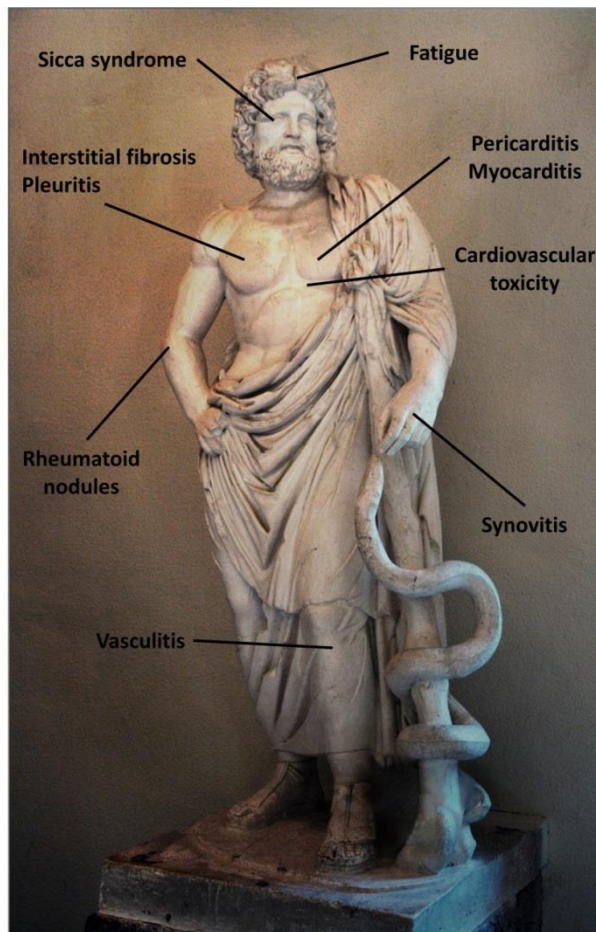


Figure 1. RA is a systemic disease, affecting several organs and having many systemic manifestations.

Picture: Statue of Asclepius (museum of Epidaurus Theatre). Modified from Wikimedia Commons (http://commons.wikimedia.org/wiki/File:Asklepios_-_Epidaurus.jpg)

1.1.4 ‘Why’ – etiology of RA, risk factors for RA

The etiology of RA remains hitherto unclear. We know however that environmental, immunological and genetic factors interact for the development of RA¹⁸. In genetically susceptible individuals, specific environmental factors can activate potentially pathogenic immune reactions, including antibody formation. It has been shown that autoantibodies are present years before the development of symptoms¹⁹. Later a stochastic factor like trauma or infection can trigger the abnormal inflammatory process. The most well-known genetic risk factors are the presence of certain HLA DR alleles (which have a common aminoacid motif, the ‘shared epitope’)²⁰ and PTPN22 gene²¹. These however are associated only with seropositive RA, with the presence of RF and ACPA antibodies in RA patients, proving how heterogeneous RA is not only on a clinical level but also on a genetic one^{22,23}.

The most well established environmental risk factor for the development of RA is smoking^{24,25}. Smoking has been shown to be a strong risk factor only for RF or ACPA positive RA, while the association is weak for seronegative disease²³. Studies have shown significant interaction between HLA-DR risk alleles and smoking in RF and ACPA positive RA patients²⁶⁻²⁸. It is also known today that RA patients who continue to smoke after the diagnosis of RA have a worse prognosis with higher risk for radiographic progression and worse response to anti-rheumatic therapy^{29,30}.

1.1.5 ‘In what way’ and ‘by what means’ – immunopathogenesis of RA³¹

The primary lesion of RA is synovitis, inflammation of the synovium in the affected joints. Immune cells ‘invade’ the normally relatively acellular synovium leading to the formation of ‘*pannus*’, a hyperplastic, and inflammatory tissue that resembles cancer tissue. Over time, this process creates a cytokine milieu in the joint that activates synovial fibroblasts and osteoclasts, which in turn degrade cartilage and bone. Key cellular mediators as well as important cytokines support this process and have been targets for the biological agents developed for the treatment of RA.

1.1.5.1 T-cells

T-cells are one of the most abundant cells in the rheumatoid synovium. The majority are CD4+ helper cells (Th). RA has traditionally been considered to be associated with an ‘immunological shift’ from Th2 to Th1, leading to a disturbance in the healthy balance between the Th derived cytokines (INF- γ from Th1 and IL-4 from Th2). Newer data however suggest that other types of Th cells could play a critical role in the pathogenesis of RA, such as the Th17 cells. Th17 cells can produce IL-17, IL-10, INF- γ , TNF, IL-6, GM-CSF. IL-17 is a highly pleiotropic cytokine which can perpetuate inflammation, promote angiogenesis and osteoclastogenesis. T regulatory cells, the so-called T-regs, are also highly represented in RA synovium and are thought to play an important role as well in the immunopathogenesis of RA.

1.1.5.2 B-cells

Special emphasis will be given on B-cells, since they are the target of rituximab, one of the biologic agents under study in this thesis.

A B-cell is a subset of lymphocytes belonging to the adaptive immune system. Their name comes from the bursa of Fabricius, the organ of maturation of B-cell in birds. In mammals the early stages of B-cell maturation takes place in the bone marrow. The major subsets of B-cells are follicular B-cells, marginal zone B-cells and B-1 B-cells, each of which is found in different anatomic locations within lymphoid tissues. Development of B-cell takes place both in the bone marrow and in peripheral lymphoid organs and involves several stages (figure 2)³². B-cells have clonally distributed antigen receptors in their surface, the B-cell receptors (BCR), which are actually membrane bound antibodies. B-cells from one clone express antigen receptors of the same specificity that are different from other clones. These antibodies, or immunoglobulins, consist of a fixed and a variable portion. There are 10^{10} different combinations of the variable portions of these immunoglobulins!

In figure 3 the structure of an immunoglobulin is shown. It comprises of two identical heavy chains (C_H) and two identical light chains (C_L). It has a

symmetric core structure. Both heavy and light chains consist of aminoterminal variable (V) regions that participate in antigen recognition. The other 'end' of the molecule is a carboxy-terminal constant (C) region that mediates effector functions.

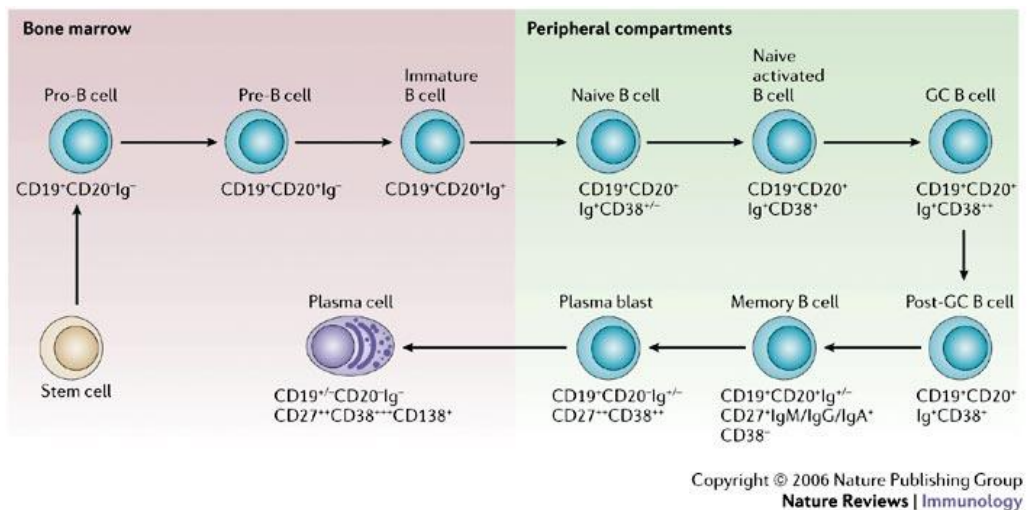


Figure 2. Stages of B-cell maturation are indicated by their anatomical site and the expression of cell-surface markers, including CD20. Copyright ©, Nature Publishing Group.

The activation and differentiation of B cells is a long and complicated process. It starts by recognition of an antigen by the B-cell receptor. The activation of other co-receptors such as CD19 and CD21 (CD=cluster of differentiation, cell surface proteins that play role in cell signaling and sometimes in cell adhesion) is required for the transmission of the first signal. In contrast to T-cells, which can only recognize small polypeptides, B-cells can recognize larger proteins. T-independent activation of B-cells occurs classically following stimulation with polyvalent antigens. Some antigens however demand cooperation with T-cells (T-dependent activation of B-cells). In this process co-stimulation pathways,

such as CD40 and CD40-ligand, as well as CD28 and CD80/CD86 (also known as B7.1/B7.2) are crucial for the initiation of the immune response. Cytokines play also a very important role in the T-cell dependent activation of B-cells, such as IL-4 and IFN γ . The type of cytokine involved in this process will influence the type of antibody isotype that will be produced by the particular B-cell. An additional cytokine required for the activation of B-cells is the BLyS (B-lymphocyte stimulator) or BAFF (B-cell activator factor of the TNF family). BAFF is produced mainly by dendritic cells and has a major role in the activation and survival of normal as well as self-reactive B-cells. After activation B-cells can differentiate into antibody-producing plasma cells or memory cells.

The multifaceted role of B-cells in the pathogenesis of RA has only recently been fully recognized. B-cells can **differentiate to plasma cells and produce autoantibodies** such as RF and ACPA. B cells are also highly capable **antigen-presenting cells** and can contribute to autoreactive T cell activation. They also **produce several cytokines**, like IL-4 and IL-10 that can promote leukocyte infiltration in the joint, angiogenesis and synovial hyperplasia. B cells can sustain **immunologic memory** by differentiating to memory B-cells. B-cells express several toll-like receptors (TLRs) on their cell surface which transmit “danger signals” by binding bacterial cell wall components or DNA (pathogen associated molecular patterns; PAMPs). Hence, hypomethylated mitochondrial DNA released from dead cells (which are abundant in RA-synovium) could conceivably activate autoreactive B-cells, driving autoantibody production and immune complex formation.

1.1.5.3 Cytokines of inflammation – the role of TNF

Cytokines in a general term are molecules involved in signaling between cells during immune response. Pro-inflammatory cytokines are important mediators of active RA promoting the activation of the adaptive immune system and the ‘communication’ between cells. T-cells, macrophages and stromal cells are the main source of cytokines in early and established RA. Interestingly, the synovial cytokine profile in patients with RA differs between those patients who will subsequently develop RA (predominance of T-cell derived cytokines such as IL-2, IL-4, IL-13, IL-17 and of stromal and macrophage derived cytokines such as

IL-1, IL-15 and EGF) and those who will go into remission or progress to a different arthritic disease. TNF (initially named TNF- α in order to be differentiated from TNF- β , now known as lymphotoxin) is produced primarily by macrophages and has many functions in the development of inflammation and the activation of other leukocytes. It is prothrombotic and promotes leukocyte adhesion and migration. It has an important role in macrophage activation and differentiation, it regulates hematopoiesis, it regulates lymphocyte development and it induces other cytokines as well. It is therefore a perfect target for RA, as it has been proven also in clinical practice.

Several other cells and mediators of both the innate and adaptive immune system are involved in the immunopathogenesis of RA, but will not be described here in more detail.

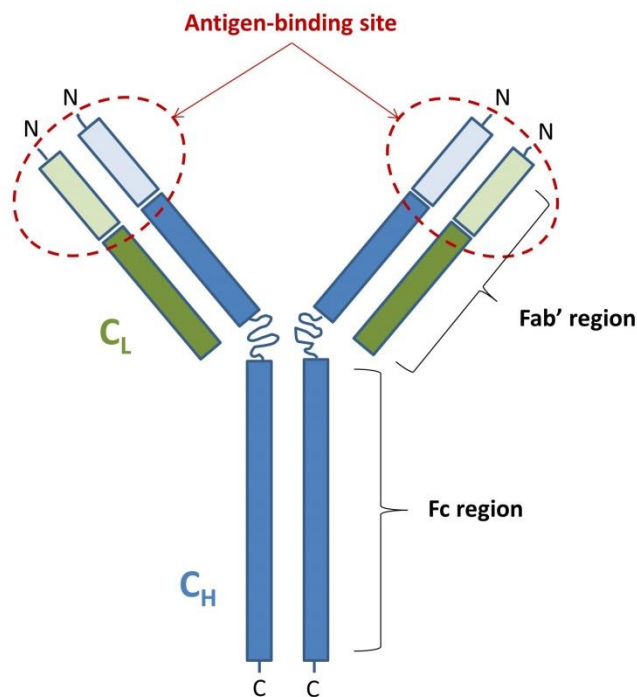


Figure 3. Molecular structure of immunoglobulin. (figure made by the author)

1.2 TREATMENT OF RHEUMATOID ARTHRITIS

1.2.1 Synthetic DMARDs³³

For an anti-rheumatic agent to be qualified as ‘disease modifying’ it needs to be able to impact the radiological progression which leads to functional decline. A DMARD should be introduced from the time of RA diagnosis, as early as possible, aiming the lowest level of disease activity. DMARDs comprise a heterogeneous group of compounds with different biochemical and pharmacokinetic properties, affecting a range of cellular targets. The discovery of synthetic DMARDs has traditionally been based on empirical data, in contrast with recently introduced biological DMARDs agents that were developed from bench to bedside. The most commonly used synthetic DMARDs in clinical practice are methotrexate, leflunomide, hydroxychloroquine, sulfasalazine and glucocorticoids. Other ones, like cyclosporine A, gold, azathioprine, D-penicillamine, are rarely used today in clinical practice.

For the purpose of this thesis, and particularly for paper II, I will go further into detail for two of the most commonly used synthetic (or conventional) DMARDs, methotrexate and leflunomide.

1.2.1.1 *Methotrexate*

Methotrexate (MTX) is a purine synthesis antagonist. It resembles folic acid (figure 4) and inhibits folate-dependent enzymes, such as dihydrofolate reductase (DHFR). DHFR inhibition leads to depletion of tetrahydrofolates that are essential for DNA, RNA and protein synthesis. MTX’s exact mechanism of action remains today, however, somewhat elusive.

MTX is an antimetabolite used in oncology and rheumatology. It was one of the first DMARDs with proven efficacy on radiographic progression. It is the cornerstone of RA treatment, even when used alone in monotherapy, but also in combination with other conventional DMARDs (leflunomide, antimalarial, corticosteroids) and biologic DMARDs (TNFis, rituximab, etc). MTX is used both orally and as an intramuscular injection once weekly in a dose ranging from 15 to 20mg in the majority of patients. Folic acid supplementation is recommended in order to reduce the risk for side effects. The latter include mainly gastrointestinal complaints and mild transaminase elevation. More rare

side effects include hematological toxicity (leucopenia, thrombocytopenia, pancytopenia), hypersensitivity pneumonitis, liver fibrosis. MTX is teratogenic and should be stopped at least 3 months before contraception.

1.2.1.2 Leflunomide

Leflunomide (figure 5) is a pyrimidine synthesis antagonist and thus multiple antiproliferative and anti-inflammatory functions. It is associated with a long half-life of about 15 days. The dose of leflunomide in RA is 10 to 20mg/day per os. Leflunomide is at least as effective as methotrexate in reducing disease burden in both early and established RA as well as in slowing radiographic progression. There are some studies that have examined the efficacy and safety of leflunomide in combination with methotrexate and with TNFis, suggesting that rheumatologists should consider these combinations. Side effects include gastrointestinal complains including diarrhea, hypertension, rash, alopecia, leucopenia. Leflunomide is also teratogenic and is strongly contraindicated in pregnant women. Due to its long half-life elimination of the drug can take up to 2 years. Washout with cholestyramine is indicated in order to facilitate clearance.

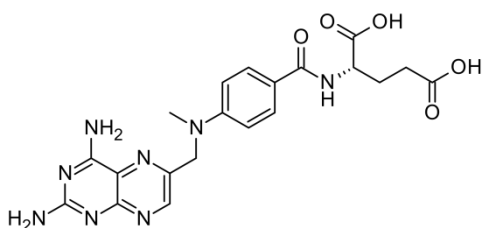


Figure 4. Chemical structure of methotrexate (source: Wikipedia, the free encyclopedia)

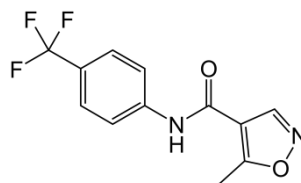


Figure 5. Chemical structure of leflunomide (source: Wikipedia, the free encyclopedia)

1.2.2 Biological DMARDs

The landscape of RA treatment has unquestionably changed dramatically during the last decade. A deeper understanding of the pathophysiological and immunological mechanisms in RA as it was presented earlier has led to the development and introduction into daily clinical practice of biologic disease-modifying agents with, in some cases, tremendous effects in patients with severe RA whose disease was difficult to control with conventional DMARDs. To date, nine biologic agents have been approved for the treatment for RA: five inhibitors of tumour necrosis factor (TNF: i.e. infliximab, etanercept, adalimumab, golimumab and certolizumab pegol), the interleukin (IL)-1 blocker anakinra, the IL-6 blocker tocilizumab, the B-cell depleting agent rituximab and the T-cell co-stimulation inhibitor abatacept³⁴. In addition, more molecules with distinct mechanisms of action are currently being tested in laboratories and in clinical trials. In all cases, very good clinical efficacy and safety were documented in trials that led to regulatory approval. Some of these data will be discussed below. The biologic agents approved today for the treatment of RA are presented in table 2.

1.2.2.1 *TNF inhibitors*

There are five TNF inhibitors available today for the treatment of RA. Although they all target the same cytokine there are important differences in their molecular structure, pharmacodynamics and pharmacokinetics that differentiate them (table 3).

The first three TNFis that received approval for the treatment of RA were infliximab, etanercept and adalimumab. Infliximab is a human murine chimeric monoclonal antibody that binds both soluble and membrane bound TNF. It is approved at a dose of 3mg/kg given at week 0, 2, 6 and thereafter every 8 weeks intravenously. Etanercept is a recombinant TNF receptor that is fused to a human Fc molecule. It is administered as a subcutaneous injection once a week. Adalimumab is a fully human monoclonal antibody against TNF. The approved dose is 40mg once every other week.

Biologic agent	Infliximab	Etanercept	Adalimumab	Golimumab	Certolizumab pegol
Molecular structure	Chimeric monoclonal antibody	Soluble TNF receptor	Human monoclonal antibody	Human monoclonal antibody	PEGylated Fab' fragment of humanized antibody
Mode of action	TNF inhibition	TNF inhibition	TNF inhibition	TNF inhibition	TNF inhibition
Approved dosage	3mg/kg every 8 week	50mg once a week	40mg every 2 weeks	50mg once a month	200mg every 2 weeks
Administration	i.v.	s.c.	s.c.	s.c.	s.c.
Biologic agent	Rituximab	Abatacept	Anakinra	Tocilizumab	
Molecular structure	Chimeric monoclonal antibody	Dimerized CTLA4 molecule	Recombinant receptor antagonist	Humanized receptor antibody	
Mode of action	B-cell depletion (binds CD20)	T-cell costimulation inhibition	Binds IL-1 receptor	Binds IL-6 receptor	
Approved dosage	1000mg x 2 *	125mg once a week (s.c.) ~750mg once a month (i.v.)	100mg once a day	8mg/kg once a month	
Administration	i.v.	i.v., s.c.	s.c.	i.v.	

Table 2. Biological DMARDs approved for the treatment of RA

The newer TNFis are golimumab and certolizumab pegol. Golimumab is a fully human IgG1 monoclonal antibody specific for both circulating and bound TNF. It is given subcutaneously once every month at a dose of 50mg and in combination with methotrexate (or another synthetic DMARD). Certolizumab pegol differs from the other antibodies as it comprises of a recombinant antigen-binding fragment (Fab') of a humanized antibody against TNF, conjugated to a polyethylene glycol (PEG) moiety. It is approved at a dose of 200mg subcutaneous injection every 2 weeks both in combination with synthetic disease modifying antirheumatic drugs (DMARDs) and as monotherapy.

Several studies have demonstrated the efficacy and safety of these TNF inhibitors in RA patients naïve to synthetic DMARDs who in practical terms often equate to newly diagnosed patients. These trials have generally provided clear evidence of the superiority of the combination of biologics with MTX over MTX alone in such patients. In a small (20 patients), randomized, double-blind, placebo-controlled trial, Quinn *et al.* showed significantly greater clinical, functional and radiological benefit of combination treatment with MTX plus infliximab in early RA compared to MTX alone³⁵. It is interesting that 1 year after stopping induction therapy with infliximab, 70% of patients had a sustained response. In a much larger study by St. Clair *et al.* patients with RA of ≤ 3 years of duration achieved significantly better clinical efficacy with infliximab plus MTX than with MTX monotherapy³⁶. Similar results were shown in large, randomized, controlled trials for etanercept and adalimumab, as well as for the newer TNFi golimumab (the data are summarized in figure 6)³⁷⁻³⁹. All of the above-mentioned studies were based on a population of patients with early RA (disease duration from 6.5 to 10.8 months) who had not received prior MTX, and they all demonstrated that the combination of a biologic agent and MTX can yield better clinical, functional and radiographic outcome than MTX alone. However, it is worth noting that a substantial proportion of patients in these trials responded well to MTX monotherapy. Thus, one should keep in mind that in the combination groups, there are patients who would have responded to MTX monotherapy as well⁴⁰.

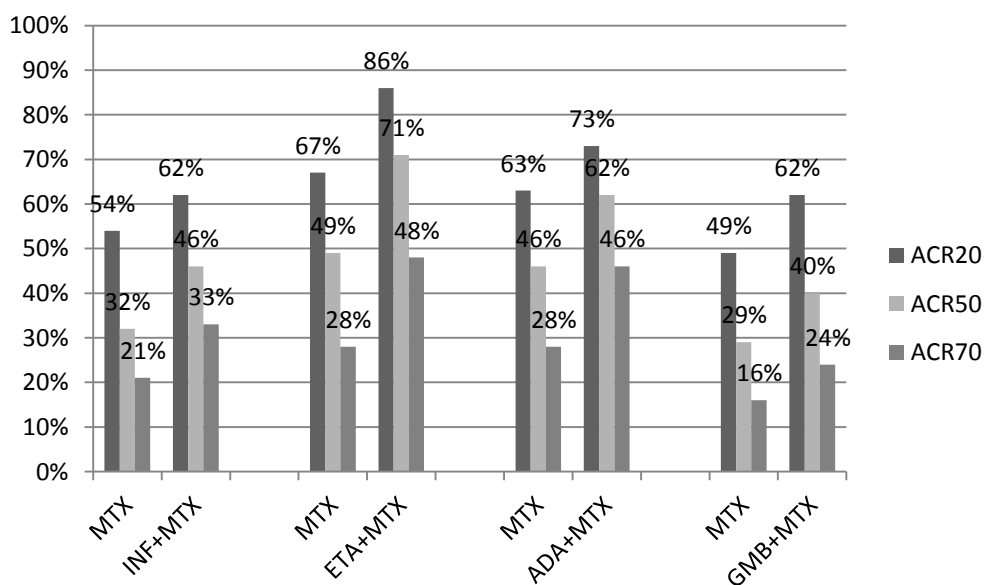


Figure 6. Efficacy of various TNFis [infliximab (INF), etanercept (ETA), adalimumab (ADA) and golimumab (GMB)] in combination with methotrexate (MTX) vs. MTX alone assessed by ACR responses in RA patients who are DMARD-naïve. Common for all TNFis was that the combination yielded significantly greater ACR responses compared to MTX monotherapy.

The most clearly established and best documented role for biologics in the treatment for RA however is in those patients who have failed to respond adequately to one or more conventional DMARD, usually including MTX. Double-blind, placebo-controlled, randomized trials for all nine biologic agents available today have been conducted and have established the efficacy of these drugs in patients with RA with inadequate response to MTX. In figure 7 the clinical responses after 6 months of therapy with all five TNFis plus MTX versus placebo plus MTX are summarized^{1,41-44}. All these biologic agents seem to have comparable efficacy in the MTX non-responder population and significantly greater efficacy than placebo.

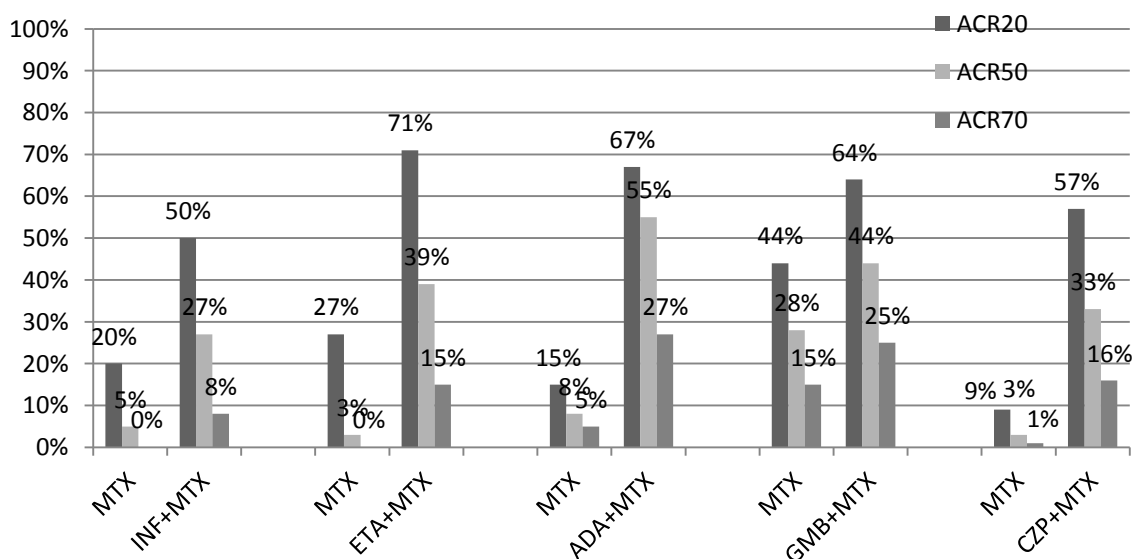


Figure 7. Efficacy of all available TNFi [infliximab (INF), etanercept (ETA), adalimumab (ADA), golimumab (GMB) and certolizumab pegol (CZP)] in combination with methotrexate (MTX) vs. MTX alone assessed by ACR responses in RA patients who were MTX non-responders. The addition of a TNFi in the treatment of these patients led to significant clinical improvement.

1.2.2.2 Rituximab

B cells play a key role in RA pathogenesis as it was shown before in detail. Their multifaceted role made them a very promising target for the treatment of RA. Today only one B-cell depleting agent is approved for the treatment of RA, rituximab. Rituximab is a genetically engineered, chimeric anti-CD20 monoclonal antibody. It comprises human IgG1Fck constant regions and small variable light and heavy chain regions from the anti-CD20 murine antibody fragment, which is reactive to human CD20. CD20 is a membrane-associated phosphoprotein that regulates the early steps in B cell activation. Its expression is restricted to B cells. Binding of CD20 by rituximab neither modulates expression nor causing substantial internalization of CD20. Treatment with

rituximab causes rapid depletion of certain B cells within the first treatment infusions and the effects can last for 6 to 9 months. CD20 positive B cell precursors, transitional B cells and naïve B cells are most susceptible to deletion by rituximab, while B1, marginal zone and germinal center B cells are more resistant.

Mechanism of action

It is hypothesized that rituximab works through numerous candidate mechanisms. These mechanisms vary depending on how the disease is expressed in each patient. The first mechanism of B cell deletion is **antibody dependent cell-mediated cytotoxicity (ADCC)**, where an antibody coated target cell is directly killed by an effector cell expressing Fc receptors. A second mechanism occurs via **complement activation through the classical pathway** which leads to the formation of the membrane attack complex (MAC) and consequently cell lysis. The third mechanism is the **induction of apoptosis**⁴⁵. Which mechanism of action takes place is influenced by host factors, such as genetic background and disease specific factors such as the availability of an intact complement pathway and the magnitude of B cell survival signals. One other possible mechanism through which rituximab might work is the **immune complex decoy hypothesis**, whereby the binding of rituximab-IgG molecules to B cells forms immune complexes that efficiently attract and find Fc gamma receptor-expressing effector cells, which diminishes recruitment of these effector cells at sites of immune complex deposition and, therefore, reduces inflammation and tissue damage⁴⁶.

By depleting B cells and modulating the immune system, rituximab leads to reduced antigen presentation, proinflammatory cytokine production and autoantibody production, thus efficiently reducing the severity of B-cell mediated autoimmune diseases, like RA.

Pharmacokinetics of rituximab, relationship between B cell depletion and clinical response

Rituximab has a mean terminal half-life of 19 to 22 days after the second infusion. Systemic clearance of rituximab is around 220 mL/d. The volume of distribution is slow at 4.3 to 4.7 L and similar to normal plasma volume. The serum concentration of rituximab appears to be inversely related to recovery of peripheral B cells.

Initially it was shown that although depletion of B cells in periphery was observed in all rituximab treated patients, not all of them responded to therapy⁴⁷. A possible explanation could be the presence of residual B cells in lymph nodes and in the synovium. Another explanation might be the sensitivity of measurement of B cells in periphery. Indeed, more recent studies have shown a correlation between the depth of B cell depletion both in the circulation and in the synovium and clinical response^{48,49}. Persistence of B cells is associated with poorer response⁵⁰.

Efficacy and safety of rituximab in RA

Rituximab is shown to be more efficacious than placebo in randomized controlled trials with an acceptable safety profile. In the SERENE trial both RTX doses (1000mg x 2 and 500mg x 2) achieved significantly greater results compared to placebo in RA patients with background MTX who had not responded adequately to MTX. No significant differences in efficacy or safety between the two doses groups were observed. The objective of the MIRROR study was to assess the efficacy of repeated treatment regimens with RTX at the same dose, 500mg x 2 and 1000mg x 2 both at baseline and after 24 weeks and at a dose-escalation, 500mg x 2 at baseline followed by 1000mg x 2 after 24 weeks again in MTX non-responders RA population. Similarly to the SERENE trial, no significant differences in response rates were shown. In the IMAGE trial, which was conducted in DMARD-naïve RA patients, both doses of rituximab were associated with significant clinical improvement (in some comparisons the improvement was slightly higher numerically for the 1000mg x 2 group but not significantly higher), but only the higher dose of rituximab was

proven effective in inhibiting progression of joint damage as assessed by the change in total Genant-modified Sharp score (mTSS) from baseline to week 52. The lower dose of rituximab could also slower the progression but the change in score compared to MTX monotherapy did not achieve the level of statistical significance.

RCT	RA population	Groups under comparison	N. patients
‘SERENE’	Biologic-naïve, MTX-non-responders	RTX 500mg x 2, RTX 1000mg x 2, PLC	509
‘MIRROR’	Biologic-naïve, MTX-non-responders	RTX 500mg x 2 at baseline and week 24 RTX 1000mg x 2 at baseline and week 24 RTX 500mg x 2 at baseline and 1000mg x 2 at week 24	314
‘IMAGE’	Early RA, MTX-naïve	MTX alone MTX + RTX 1000mg x 2 MTX + RTX 500mg x 2	755
‘DANCER’	MTX and biologic non-responders	Placebo * RTX 1000mg x 2 * RTX 500mg x 2 *	465
‘REFLEX’	TNFi non-responders	Placebo RTX	520

Table 3. Five large RCTs conducted during the last years with the aim to assess the efficacy and safety of RTX in RA.

In the DANCER trial rituximab proved to be effective in patients who had not previously responded to DMARD treatment, including biologic DMARDs. Both rituximab dosages were effective, with 54–55% of patients achieving an ACR20 response by week 24, and a high proportion achieving ACR50 or ACR70 responses. No dose-response relationship was established, based on the ACR20

criteria, for the 2 rituximab doses studied. There were, however, trends to indicate that the dose may influence the achievement of high-level response (i.e., ACR70 response and good EULAR response). Similarly, in the REFLEX study RTX yielded significant ACR responses compared to placebo in patients with longstanding disease who did not respond to TNFis. The above trials are summarized in table 3 and figure 8. In an indirect comparison one could observe that the efficacy of rituximab is best in DMARD-naïve patients and reduced in patients who have already failed at least one biologic agent.

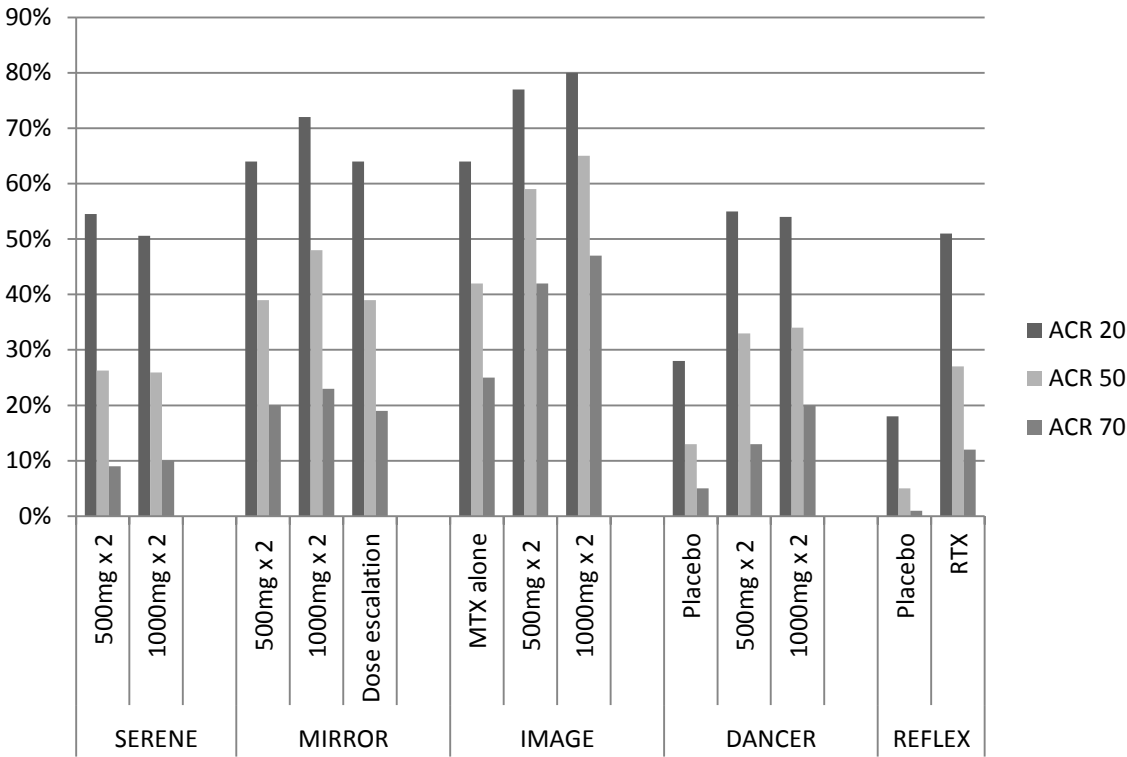


Figure 8. Efficacy of rituximab as assessed by ACR responses in several RA populations and in different doses in five large RCTs.

All the above studies assessed the safety of rituximab in a systematic way. The most common adverse effects of rituximab are infusion reactions (common with the first infusion), increased risk for infections (upper respiratory tract infection, urinary tract infection, nasopharyngitis), reactivation of viral infections. Hitherto few cases of progressive multifocal leukoencephalopathy (PML) have been reported in RA. In a pooled analysis of safety data from patients treated with rituximab in combination with methotrexate in a global clinical trial program, the overall rate of adverse events was 357 events per 100 patient-years (95% CI 354.4; 364.9). The rate of adverse events seems to decline with time. Hypoglobulinemia is an expected finding in rituximab treated patients. IgM levels decrease with multiple courses of rituximab, but this does not appear to be associated with increased risk for serious infections. IgG is the most important among serum immunoglobulins for protective immunity. In some rituximab treated patients levels of IgG can also be decreased but to a minimal degree compared to IgM. No obvious differences in the rate of adverse events have been observed between the higher and the lower dose rituximab.

1.2.3 Treatment strategies

1.2.3.1 When should a biologic agent be introduced?

The main conclusion from the above studies was that TNF inhibition can lead to significant clinical and functional improvement as well as inhibit radiographic progression in RA patients both before the introduction of a synthetic DMARD, like methotrexate, and after the inadequate response to at least one synthetic DMARD. These results do not, however, answer the question of whether a combination of biologic drugs with MTX is also superior to a combination of synthetic DMARDs. So far, only a few clinical trials have made a direct comparison of these two treatment options, by adding a biologic agent or adding/switching to another synthetic DMARD. In the SWEFOT trial, patients with early RA with an inadequate response to MTX after 3 months, defined as lack of achievement of low disease activity, were randomly allocated to addition

of either sulfasalazine and hydroxychloroquine or infliximab⁵¹. The latter group had significantly greater responses after 12 months of therapy, with 39% of patients achieving the primary end-point EULAR good response compared to 25% in the former group ($P = 0.016$). However, after 2 years the clinical difference was smaller and no longer statistically significant⁵². Radiological progression was, however, greater with conventional therapy than with the biologic agent. In the BeSt trial it was shown that initial combination therapy with initial high-dose prednisone followed by a gradual prednisone-dose reduction or with infliximab provided earlier clinical improvement than sequential monotherapy and conventional combination therapy⁵³. After 2 years, patients in all four treatment groups had approximately the same improvement in disease activity and functional status irrespective of initial treatment, probably because of tight control and frequent treatment adjustments. However, the more aggressively treated patients had less radiological progression of joint damage, and during the second year, more of them could be treated successfully with monotherapy, suggesting that the initial aggressive therapy did result in some long-term gains.

In the Treatment of Early Aggressive RA (TEAR) trial, patients with early RA were randomized to one of four groups: methotrexate monotherapy followed by triple therapy in case of insufficient response ($n = 124$); methotrexate followed by the addition of etanercept ($n = 255$); immediate triple therapy ($n = 132$); or immediate methotrexate plus etanercept ($n = 244$)⁵⁴. Rather surprisingly, at 1 year follow-up none of the four strategies was superior clinically, and only small differences were observed in radiographic progression. In a recently published double-blinded, randomized clinical trial, patients with established RA with an average disease duration of 5 years who had been receiving methotrexate in at least one year but despite that had still active disease, were randomized to receive either triple therapy (methotrexate, sulphasalazine and hydroxychloroquine) or methotrexate + etanercept⁵⁵. The main result was that triple therapy was non-inferior to the addition of a biologic agent to methotrexate. The difference in average 28-joint disease activity score (DAS28) at 24 weeks almost achieved significance ($P = 0.06$), but was smaller than the non-inferiority margin. Thus, one could summarize the result by saying that triple therapy might be a little bit less effective than the addition of an anti-TNF

agent in the event of inadequate response to traditional DMARD therapy, but the difference is not clinically relevant⁵⁶.

1.2.3.2 What happens after the failure of the first TNFi?

Data from randomized trials and cohort studies suggest that about one-third of patients discontinue therapy with a first biologic within a year because of primary ineffectiveness, loss of efficacy after a period of time or intolerance. The goal of treatment for these patients remains remission. Even patients with moderate responses ('partial responders') should eventually be candidates for an alternative treatment, taking into account that even 'smoldering' disease activity might lead to structural damage.

After the failure of one biologic agent for the reasons described above, and after having perhaps tried to modify the dose of the concomitant DMARDs with no effect, three main treatment options are available: (i) optimize the dose of biologic drug; (ii) switch between TNF inhibitors; or (iii) switch to a biologic agent with a different mechanism of action (figure 9).

Optimizing the dose

Controversial data are available concerning the optimization of the infliximab dose. In the ATTRACT trial, four different treatment regimens (i.e. infliximab 3 mg/kg every 4 and 8 weeks, and 10 mg/kg every 4 and 8 weeks) yielded similar American College of Rheumatology (ACR) responses at 24 weeks⁴¹. At 48 weeks, however, there was a tendency for the lowest dosage of infliximab to be less effective than the higher ones, but this difference was only significant with respect to the ACR50 responses⁵⁷. Results from uncontrolled observational studies have suggested that in patients with secondary loss of efficacy to infliximab, a higher dosage of infliximab could provide better efficacy⁵⁸. On the other hand, in a double-blind randomized trial, Pavelka *et al.* showed no significant difference in efficacy of two dosages of

infliximab (3 and 5 mg/kg) after initial failure of the lower dosage to lead to remission⁵⁹. Moreover, the higher dosage had a poorer safety profile. Another large trial of infliximab showed similar results, with no significant superiority of the higher dosage of 6 mg/kg³⁶. In an observational study conducted in our centre, patients in whom the dose of infliximab was increased in clinical practice appeared to have a benefit, as defined by reduction in the disease activity score (DAS28)⁶⁰. However, patients in the control groups (i.e. patients with no change in infliximab dose and those receiving a stable dose of etanercept) also showed an improvement in DAS28. This observation suggests that the improvements were most probably attributable to regression to the mean and that no important benefit is gained from dose increases of infliximab. Finally, van den Bemt *et al.* found that 17 of 18 patients who were in clinical practice treated with infliximab at dosages higher than 3 mg/kg showed no deterioration of their RA if the dosage was reduced to 3 mg/kg⁶¹. In conclusion, the evidence suggests that increasing the dose of infliximab might result in loss of time, higher cost and potentially more side effects with no significant efficacy gain in most patients. Therefore, it would clearly be useful to be able to identify, using relevant biomarkers, a smaller subset of patients who might truly benefit from dose increases. Studies to investigate this possibility are currently underway.

Switching between TNF inhibitors

Different TNF inhibitors might target the same cytokine but differ substantially in their molecular structure and immunological actions. This is the rationale behind switching between different TNF inhibitors. Because of differences in pharmacokinetics between these inhibitors, it is possible that a patient will respond to an alternative agent after the failure of the first. This issue was investigated in the randomized double-blinded GO-AFTER study, in which after failure of a prior TNF inhibitor, patients who received golimumab showed significantly greater responses than those who received placebo⁶². In addition, results from many observational studies support switching between TNF inhibitors, as a substantial proportion of patients can benefit from this strategy⁶³⁻⁶⁹. Cohort study data also suggest a gradual loss of efficacy after a greater number of switches. Thus, a first switch might provide significant

improvement, whereas the effect is much less profound at the second or third switch.

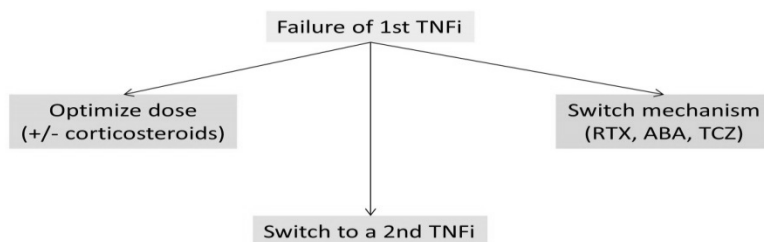


Figure 9. Treatment options after the failure of the 1st TNFi.

Switching mechanism of action

After the failure of TNF inhibition (with a single or multiple agents), the next step is change of mechanism, which might be more logical than trying different regimens of the same drug class. Large trials have proved the efficacy of rituximab, abatacept and tocilizumab versus placebo after TNF treatment⁷⁰⁻⁷². Superiority of rituximab over placebo was observed in the REFLEX trial. Abatacept demonstrated acceptable safety and clinically meaningful efficacy in patients who failed TNF inhibitor treatment in the ATTAIN trial. Additionally, in the RADIATE trial, tocilizumab-treated patients achieved significantly better results than those who received placebo during the first 6 months of therapy. But are these biologic agents with a different mechanism of action better than an alternative TNF inhibitor? Again, no randomized clinical trial has provided us with hard evidence to answer this question. On the other hand, observational studies have compared the two treatment options. In the Swiss Clinical Quality Management program for RA (SCQM-RA) registry, patients with inadequate response to TNF inhibitor treatment achieved greater

reductions in DAS28 when switching to rituximab than to an alternative TNF blocker⁷³. In a sub-analysis of the same population, it was shown that the superiority of rituximab over an alternative TNF inhibitor was observed for the subgroup of patients who discontinued previous TNF inhibitor therapy because of primary or secondary inefficacy⁷⁴. Newer data from the British Society for Rheumatology Biologics Register suggest that switching to RTX may be of more benefit than switching to an alternative anti-TNF therapy after failing the first anti-TNF therapy in RA patients⁷⁵. In a prospective observational study from Spain no difference in the reduction of DAS28 was observed during the first year of treatment between RTX and TNFi groups, but in a sub-analysis the difference was different between adalimumab/infliximab and RTX⁷⁶.

1.2.3.3 When can a biologic treatment be discontinued?

After the achievement of low disease activity or remission, the next goal is the ‘biologic-free remission’, which is important with respect to long-term safety issues, patient comfort and health economics. In various settings, the possibility has been investigated of discontinuing the biologic agent whilst maintaining the patient in remission on a conventional DMARD. As part of the ATTRACT study⁵⁷, 17 patients in a single center in the United Kingdom received infliximab and all 17 experienced flare-ups after discontinuation of the biologic therapy after 2 years, with a mean time of 13.5–15.0 weeks after the end of therapy. Of importance, reintroduction of infliximab after disease flare was associated with comparable responses without any safety issues. Whereas patients included in the ATTRACT study had longstanding disease (mean disease duration, 11 years), Quinn et al. addressed the same question in a randomized, double-blind, placebo-controlled trial in a population of patients with early RA, with symptom duration of <12 months³⁵. These authors showed that induction of remission with infliximab plus MTX in early, poor prognosis RA provided not only significant reduction in synovitis and erosions at 1 year (shown by magnetic resonance imaging) but also sustained functional and quality-of-life benefits for 70% of the patients at 2 years despite infliximab withdrawal. More recently,

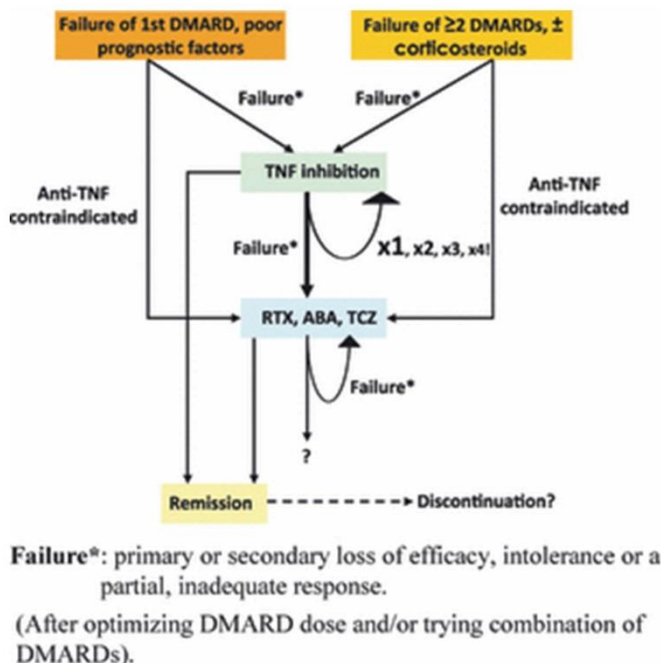
Tanaka et al. determined the possibility of discontinuing infliximab after attaining DAS-guided low disease activity in patients with RA in the remission induction by infliximab in RA (RRR) study⁷⁷. Of 102 patients, 56 (55%) maintained DAS28 < 3.2 and 44 (43%) reached remission (DAS28 < 2.6) 1 year after the discontinuation of infliximab. The mean disease duration in this study was 5.9 years, which suggests that discontinuation of infliximab would be possible not only in patients with early RA but also in patients with more established disease. In a post hoc analysis from the BeSt study, it was shown that significantly more patients who received initial combination therapy with infliximab and MTX achieved sustained DAS \leq 2.4 and were able to discontinue infliximab, compared with those with delayed introduction of the biologic agent (56% vs. 29%, $p = 0.008$)⁷⁸. It was also shown in the BeSt study that the shorter the symptom duration, the higher the likelihood of a biologic-free, and even a drug-free, remission⁷⁹. In the OPTIMA trial RA patients with early RA who achieved stable low disease activity on adalimumab plus methotrexate who withdrew adalimumab mostly maintained their good responses⁸⁰. Even in more established RA, discontinuation of adalimumab can be feasible but mainly for patients on deep remission, as shown in the HONOR study⁸¹.

The results of a systematic review and meta-analysis showed that patients with established RA who stopped treatment with synthetic DMARDs had a significantly higher risk of disease flare or deterioration than those who continued treatment⁸². In this analysis, however, patients had RA of more than 2 years of duration.

From the results of the above studies, one can draw several conclusions. First, biologic-free remission might be possible after achieving remission or low disease activity in a considerable proportion of patients. Second, the duration of disease until the introduction of the biologic treatment might be negatively associated with the risk of deterioration after discontinuation of treatment, thus suggesting that earlier initiation of biologic treatment not only leads to better results but also increases the possibility of withdrawal of biologic agents with

maintenance of remission. Third, if a patient has had a remission for a long time, it is more likely that the patient will remain in remission.

In figure 10 an algorithm for biologic treatment of RA is shown below.



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<http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2796.2011.02355.x/full#ft3>

Figure 10. Algorithm for biologic treatment of RA.

1.3 ASSESSMENT OF THERAPY IN RHEUMATOID ARTHRITIS

1.3.1 General rules

Clinicians use both implicit and explicit criteria for the assessment of a therapy and follow-up of patients. An example of an implicit criterion is ‘the patient feels better’, while an explicit criterion will be more precise and would be comparable between different subjects, for example visual analogue scale (VAS) for pain (0 to 10). It is obvious that for research purposes but also for the clinical practice good explicit criteria are needed for the monitoring of therapy outcomes.

The general acceptable goal of RA treatment today is **remission or low disease activity** for patients for whom remission is not possible⁸³. **Tight control** with regular disease assessments and treatment to target are crucial in order to follow treatment results and if the goal is not achieved to take further actions. This strategy can lead to better results, as it has been shown in studies such as the Best trial and the TICORA trial^{84,85}. Of vital importance is however the definition of the goal, and in this case remission.

There are three important aspects of response to therapy in RA today: disease activity, function and damage. All three must be taken into consideration when assessing a treatment. Consequently, we need assessment measures for disease activity, function and damage.

1.3.2 Tools

1.3.2.1 Assessment tools for disease activity

RA’s heterogeneity makes no single instrument able to describe the disease activity equally well for each patient. And this is obvious from the clinical reality, where some patients have many swollen and tender joints with no or minimal elevation of acute-phase reactants (ESR and CRP), while for other patients it is the other way around. For this reason composite measures are to prefer from single ones. A composite score is a combination of various single measurements but with minimum overlap. Composite scores used in RA are

shown in table 4. The most widely used of them is the DAS28 (disease activity score based on 28 joints)⁸⁶. There are two separate formulas for ESR and CRP⁸⁷. Another composite score commonly used is the Simplified Disease Activity Index (SDAI), which is the sum of the 28-SJC, the 28-TJC, and the patient and investigator global assessments of disease activity on a 10 cm VAS and CRP in mg/dl⁸⁸. The Clinical Disease Activity Index (CDAI) is a modification of the SDAI without the laboratory parameter CRP to allow immediate clinical assessment^{89,90}. According to the level of DAS28, disease activity can be categorized to high, moderate, low or remission (figure 11).

Composite scores used for the assessment of RA disease activity	
DAS28	$0.56\sqrt{\text{TJC28}} + 0.28 \sqrt{\text{SJC28}} + 0.70 \cdot \ln(\text{ESR}) + 0.014 \cdot \text{VAS GH (Pat)}$
DAS28 (CRP)	$0.56\sqrt{\text{TJC28}} + 0.28 \sqrt{\text{SJC28}} + 0.36 \cdot \ln(\text{CRP} + 1) + 0.014 \cdot \text{VAS GH (Phy)} + 0.96$
SDAI	$\text{SJC28} + \text{TJC28} + \text{VAS GH (Pat)} + \text{VAS GH (Phy)} + \text{CRP}$
CDAI	$\text{SJC28} + \text{TJC28} + \text{VAS GH (Pat)} + \text{VAS GH (Phy)}$

Table 4. Composite scores for the assessment of disease activity in RA.

To better describe the effectiveness of a treatment it is important to have information not only about the level of disease activity achieved (at several time points after baseline), but also about the degree of improvement, the response. The two most widely accepted measures of response are the EULAR response criteria (table 5A) and the ACR response criteria (table 5B)⁹¹⁻⁹⁴.

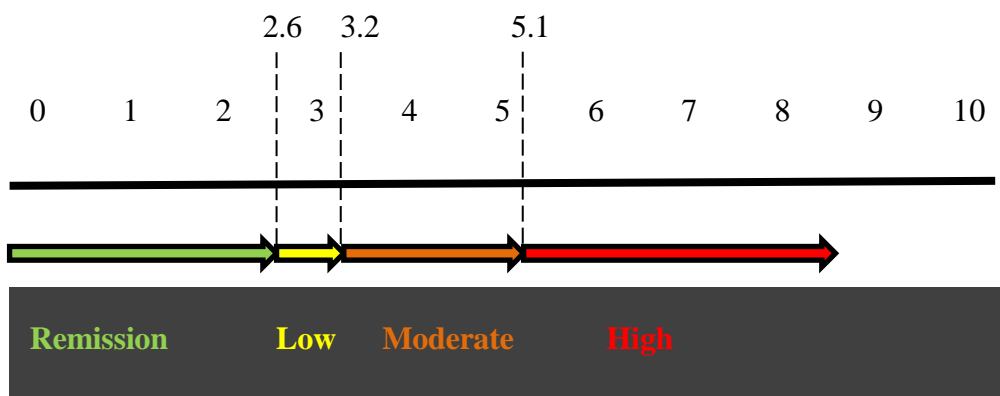


Figure 11. Disease activity state based on DAS28.

Improvement in DAS28 from baseline			
DAS28 at endpoint	≥ 1.2	>0.6 and <1.2	≤ 0.6
≤ 3.2	GOOD	MODERATE	NO
$5.1 \leq$ and <3.2	MODERATE	MODERATE	NO
>5.1	MODERATE	NO	NO

Table 5A. EULAR response criteria based on DAS28 status and DAS28 improvement from baseline.

ACR 20	ACR 50	ACR 70
20% improvement in <ul style="list-style-type: none"> • TJC AND • SJC and 20% improvement in 3 of the following: <ul style="list-style-type: none"> • VAS pain • VAS GH (Pat) • VAS GH (Phy) • ESR or CRP • HAQ 	50% improvement in <ul style="list-style-type: none"> • TJC AND • SJC And 50% improvement in 3 of the following: <ul style="list-style-type: none"> • VAS pain • VAS GH (Pat) • VAS GH (Phy) • ESR or CRP • HAQ 	70% improvement in <ul style="list-style-type: none"> • TJC AND • SJC And 70% improvement in 3 of the following: <ul style="list-style-type: none"> • VAS pain • VAS GH (Pat) • VAS GH (Phy) • ESR or CRP • HAQ

Table 5B. ACR response criteria.

1.3.2.2 Assessment tools for function

The main assessment tool for the evaluation of function in RA is the Health Assessment Questionnaire (HAQ)^{95,96}. This disability index comprises of 20 questions regarding the ability of patients to perform 20 every-day activities. The score ranges from 0 to 3.

1.3.2.3 Assessment tools for damage

Several imaging techniques are available today in research and in clinical practice for diagnosis and treatment follow-up. Conventional radiography remains today the most widely implemented imaging tool for the evaluation of damage caused by inflammation in RA. Damage is closely related both to activity and function.

The information from radiographs has to be quantified in order to be able to be comparable both between different subjects but also for the individual patient

over time. Several scoring methods have been developed. In this thesis (paper VIII) the Shard/van der Heijde scoring method was used for the evaluation of radiographical progression⁹⁷.

2 OBJECTIVES

2.1 OVERALL OBJECTIVE

RCTs led to the approval of several biological agents for the treatment of RA. Most of these agents have been used by rheumatologist in an increasing rate the last years. However the use of biological agents is far from being optimized, since there are still many unanswered questions, for example:

- 1) When should a biologic agent be introduced?
- 2) After the failure of a TNF inhibitor (which is usually the first line biologic unless contraindicated) what is the best second-line biologic? Can an alternative TNF inhibitor be used, and in that case plays the type of TNF inhibitor any role? Or would switching of mechanism of action lead to better results?
- 3) Could a biologic be discontinued after achieving the goal (remission or low disease activity)?
- 4) Are there ‘target groups’ of patients who have a higher chance to respond to a specific biologic? Are there predictors of response to specific agents?

Guidelines and protocols are needed for a better utilization of these highly potent and expensive treatments. But while RCTs remain the ‘gold standard’ today and provide the most reliable answers to specific questions the more complex questions that govern clinical decision making can rarely be addressed adequately using RCTs. But even if some of these questions could be addressed through RCTs it would demand a lot of time and money.

Additionally, RCTs usually have strict inclusion and exclusion criteria, and thus they examine aspects of treatment in a very specific RA population that do not represent reality. This is the so called **external validity** of RCTs that is low compared to observational studies. Today we have at our disposal a well-structured follow-up system, the registries, which when properly used can provide us with very useful and important information that RCTs cannot do, for the reasons stated above, and thus completing the puzzle.

2.2 SPECIFIC OBJECTIVES

2.2.1 Part I: Studies of the treatment of RA with rituximab (papers I-IV)

As it was presented earlier, the efficacy and safety of rituximab has been well demonstrated in RCTs. However, several important questions that relate to the practical use of rituximab in RA need to be further elucidated, and they were the objectives in the first four papers:

I) Paper I

- a. Characterization of a large international cohort of RA patients treated with rituximab.
- b. Effectiveness of treatment in a real-life RA population.
- c. Possible predictors of response to therapy with rituximab.
- d. Off-label use of rituximab, for example as first line biological treatment.

II) Paper II

- a. Description and comparison of the effectiveness and safety of rituximab as monotherapy or in combination with synthetic DMARDs such as methotrexate and leflunomide.

III) Paper III

- a. Comparison of the effectiveness of two different doses of rituximab, the approved dose rituximab (1000mg x 2) and a lower dose (500mg x 2) in practice.

IV) Paper IV

- a. Characterization of the rate of retreatment with rituximab in every day clinical practice.
- b. Assessment of the feasibility of achieving further clinical improvement with repeated treatment cycles.
- c. Comparison of the effectiveness of two retreatment strategies: on-flare and fixed.

2.2.2 Part II: Studies on switching between biologics, on TNF inhibition and discontinuation of TNFis (papers V-VIII)

The specific aims of the studies were:

V) Paper V

- a. Assessment of switching from first to second TNFi under different circumstances (according to the reason for discontinuation and the type of the first TNFi) trying to identify an optimal switching strategy.
- b. Description and comparison of the drug survival of adalimumab, etanercept and infliximab as 2nd TNFis after switching from a 1st TNFi in patients with RA.

VI) Paper VI

- a. Description and comparison of the effectiveness of an alternative TNFi or RTX for patients who failed one TNFi.
- b. Whether the type of switch (type of first TNFi) and reason for discontinuation of the previous agent can affect the results.

VII) Paper VII

- a. Description of the use of certolizumab pegol in RA in a real-life setting.
- b. Assessment of the effectiveness of treatment both in a biologic naïve population and in patients who have previously failed at least one biologic agent.
- c. Assessment of drug survival.

VIII) Paper VIII

- a. Assessment of the feasibility of discontinuing adalimumab treatment while maintaining remission in RA patients with established disease who are in stable remission (defined as DAS28 < 2.6 for ≥ 3 months) on combination therapy with adalimumab + methotrexate (MTX).

3 MATERIALS AND METHODS

This thesis is based on eight epidemiological studies. The term **epidemiological** is to characterize the type of research performed on human subjects (etymology: ‘epi’ = upon and ‘demos’ = people and ‘logos’ = study) in contrast to experimental studies. Epidemiological studies can be further categorized to observational studies and clinical trials. In observational studies there is no intervention and the subjects are observed prospectively or retrospectively.

The first seven papers are **cohort observational studies**. The definition of a cohort study is the analytic method of epidemiological study in which subsets of a defined population are identified who are, have been, or in the future may be exposed or not exposed, or exposed in different degrees, to a factor or factors hypothesized to influence the probability of occurrence of a given disease or other outcome. The cohort studies presented here are based on large regional, national and international registers of RA patients treated with biologic DMARDs.

3.1 REGISTERS USED

3.1.1 The CERERRA cohort (papers I-IV)

In 2010 a European collaboration between registries from 10 different European countries, the European Collaborative Registries for the Evaluation of Rituximab in RA (CERERRA) initiative, was initiated. This is a collaboration between European registries from the Czech republic, Denmark, Finland, the Netherlands, Norway, Portugal, Russia, Slovenia, Spain, Sweden, and Switzerland. Romania joined the collaboration 2 years ago (figure 12).

Approximately once per year each of the participating countries contribute fully anonymized data of RA patients who are or have been treated with rituximab. The type of information collected is based on predefined specific objectives for specific projects.

The datasets from each country were collected and after a second quality control (the first being conducted by the responsible person in each country) they were organized in a central dataset which was the cohort.

The main data that were collected were:

- 1) Demographic information: age, sex
- 2) Disease-specific information: disease duration, RF and anti-CCP status (defined as positive or negative according to each laboratory).
- 3) Effectiveness data: SJC, TJC, ESR, CRP, VAS pain, VAS general health (patient), VAS general physician, DAS28-ESR, DAS28-CRP, HAQ for baseline and for each follow-up visit.
- 4) Treatment information: rituximab dose, date of rituximab initiation, date of rituximab discontinuation (if available), date and dose of rituximab retreatment and when available even reason for retreatment, number of prior synthetic and biologic DMARDs used, concomitant synthetic DMARD and corticosteroids used as well as dose.

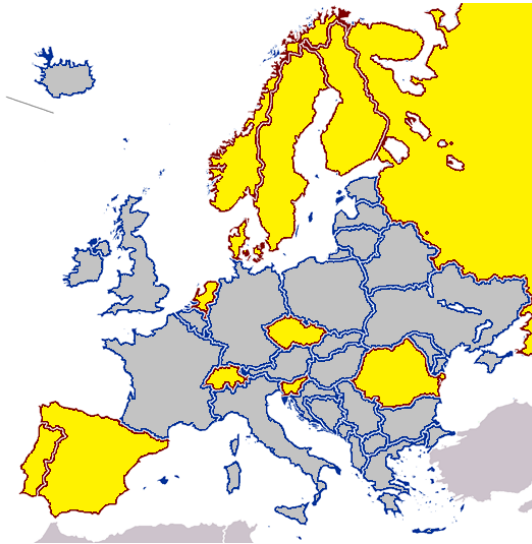


Figure 12. Countries (in yellow) participating in the CERERRA collaboration

3.1.2 The STURE register (paper VI)

The Stockholm TNF follow-up registry (STURE) database collects efficacy data for all patients starting biological treatments at the major hospitals in Stockholm, as part of the nationwide registry of Anti-Rheumatic Therapy in Sweden (ARTIS). The design of the study was approved by local ethical committees. The assessments are performed at baseline, 3, 6, and 12 months, and annually thereafter, and include the American College of Rheumatology (ACR) core outcomes [the 28 swollen (SJC) and tender joint count (TJC), visual analogue scales (VAS) for global health and for pain, the Health Assessment Questionnaire (HAQ) disability index, erythrocyte sedimentation rate (ESR), C-reactive protein], the DAS28 score and record of concurrent medications.

3.1.3 The ARTIS register (papers V and VII)

The ARTIS register is the nationwide Swedish Biologics Register (Anti-Rheumatic Therapy in Sweden (ARTIS)). To this register, data on adult patients prescribed biologic agents for the treatment of rheumatic diseases in Sweden have been collected since 1999. The coverage of the ARTIS database has been estimated to be nearly 90% of all eligible patients with RA^{98,99}.

3.2 A PILOT CLINICAL TRIAL (PAPER VIII)

The last study is based on a multi-center, randomized, controlled, open-label, pilot study. The main inclusion criteria were: age ≥ 18 ; diagnosis of RA based on 1987 ACR classification criteria and positive RF or at least one erosion on the radiograph of hands or feet; treatment with adalimumab in the approved dose of 40mg every other week for at least 6 months; concomitant treatment with MTX in a dose of at least 10mg per week for a minimum of 6 months (stable dose for a minimum of 3 months); stable DAS28 remission ($\text{DAS28-ESR} \leq 2.8$) for at least 3 months based on assessments at baseline and on at least one more occasion 3-6 months prior to baseline, documented in patient record or registry. Concomitant corticosteroids were allowed if the dose was 10mg per day or less (prednisolone or equivalent) and has been stable for at least 3 months at study entry.

3.3 STATISTICAL ANALYSIS

3.3.1 Descriptive statistics

Appropriate parametric and non-parametric statistical tests were used for the description of normally and non-normally distributed continuous numerical variables. The normality of variables was tested by skewness and by Kolmogorov-Smirnov test at certain studies. Variables which were similar to normal distribution were presented as mean \pm SD, while non-normally distributed variables were presented as median with interquartile range (IQR). Student t-test was used to compare two normally distributed continuous variables, while one-way analysis of variance was used to compare three or more groups, followed by Bonferroni test for post hoc comparisons between the groups. Chi square (χ^2) test was used to compare nominal variables. Kruskal–Wallis test was used to compare non-normally distributed variables. The level of statistical significance was set to 5%.

3.3.2 Logistic regression analysis

Logistic regression analysis is a type of statistical model used to predict a binary response (for example good response to therapy or not). Univariate is when one independent variable is used, while multivariate when many independent factors are tested at the same time. In order to know which variables to include in the multivariate model, we started with univariate analysis separately for several demographic and disease variables. The results from these analyses ($p < 0.25$ as the criterion) and correlation analyses (Pearson and Spearman correlations) guided the selection of variables for the multivariate logistic regression analyses with dichotomized responses as the dependent variables. Age and sex were usually included in the multivariate analyses. The non-significant variables were removed by stepwise backward selection. We then added back into the models, one at a time, any variable not originally selected from the univariate analyses. The variables were kept in the models if significant. Appropriate tests for linearity, interactions and goodness of fit were performed.

3.3.3 Mixed model analysis

A mixed effects model is a type of regression model that takes into consideration variation that is not generalisable to the independent variables. Such variables might include variation across different countries, different centers, different assessors, etc. These individual differences can be modeled by assuming different random intercepts for each subject. In the mixed model one or more random effects are added in the fixed effects. *The mixture of random and fixed effects is what makes the mixed model a 'mixed' model.*

Mixed effects model allows continuous dependent and independent variables as well as interactions between any combination of discrete and continuous variables. They are particularly useful in settings where repeated measurements are made on the same statistical unit, as in longitudinal studies. Because of their advantage to deal with missing values, mixed effects models are often preferred over more traditional approaches such as repeated measures ANOVA.

4 RESULTS

4.1 PAPERS I-IV: ASPECTS OF RITUXIMAB USE IN RHEUMATOID ARTHRITIS

4.1.1 Paper I

Characterization of the cohort

Patients included in this analysis reflected a typical RA population with established disease. The total number of patients was 2019. The mean (SD) age and RA disease duration was 53.8 (13.3) and 12.1 (8.9) years, respectively. 86% of patients were RF positive and 77% were anti-CCP positive. Patients had failed a mean of 2.7 prior synthetic DMARDs and 1.1 prior biologic DMARDs. Interestingly more than one third of patients were biologic-naïve. This is off-label, since rituximab is approved for patients that do not respond to TNF inhibition. Concomitant synthetic DMARDs were used by 76.7% of the patients. There was significant heterogeneity between the countries for several baseline characteristics.

Effectiveness of treatment and predictors of response

Disease activity based on DAS28 decreased significantly during the first 6 months of treatment. EULAR good/moderate responses at 3 months were achieved by 195/483 out of 1087 patients (17.9%/44.4%) and by 210/402 out of 945 patients (22.2%/42.5%) after 6 months. DAS28 improvement of >1.2 was observed in 62.5% of patients. Seropositive patients (based on RF and/or anti-CCP status) achieved significantly greater clinical responses at 3 and 6 months compared to seronegative patients. Patients who received rituximab as first biologic or after the failure of 1 TNFi responded significantly better compared to those who had failed two or more prior TNFis. In a multivariate logistic regression analysis model anti-CCP positivity, lower number of prior DMARDs and lower DAS28 at baseline were independently associated with EULAR good response to rituximab therapy after 6 months from baseline (table 6).

Variable	OR (95% CI)	p-value
Univariate adjusted analysis		
N. prior synthetic DMARDs	0.84 (0.75-0.94)	0.002
N. prior biologic DMARDs (0-1 vs. ≥ 2)	1.73 (1.25-2.38)	0.001
Baseline HAQ	1.20 (0.80-1.70)	0.30
Baseline DAS28	0.90 (0.87-1.02)	0.11
Disease Duration	0.99 (0.98-1.01)	0.48
RF (pos vs. neg)	1.50 (1.01-2.36)	0.04
Anti-CCP (pos vs. neg)	3.00 (1.56-5.77)	0.001
Conc. DMARDs	0.95 (0.67-1.33)	0.75
Conc. glucocorticoids	0.77 (0.57-1.04)	0.09
Multivariate adjusted analysis		
Anti-CCP (pos vs. neg)	2.86 (1.43-5.71)	0.003
N. prior synthetic DMARDs	0.84 (0.70-1.01)	0.06
N. prior biologic DMARDs (0-1 vs. ≥ 2)	1.89 (1.02-3.51)	0.04
Baseline DAS28	0.74 (0.61-0.91)	0.003

Table 6. Adjusted (for age and sex) univariate and multivariate logistic regression analysis with EULAR good response at 6 months as dependent variable and several baseline disease and treatment characteristics as independent variables. .

4.1.2 Paper II

In this study 2265 RA patients who started treatment with rituximab were included. The majority, 1195 patients, received concomitant methotrexate, while 177 were treated with with rituximab plus leflunomide and 505 with rituximab alone. The mean dose (\pm SD) of methotrexate was 14.4 ± 5.4 mg a week. The majority of patients on rituximab plus leflunomide (95.1%) were treated with 20 mg leflunomide a day (mean \pm SD daily dosage 17.6 ± 3.6 mg). Patients on rituximab plus leflunomide achieved a greater reduction in disease activity score in 28 joints (DAS28) values from baseline to the 6 and 12-month assessments compared with rituximab plus methotrexate and rituximab alone. Significantly more patients achieved a EULAR good response at 6 and 12 months when treated with rituximab plus leflunomide compared with rituximab plus methotrexate and rituximab alone (figure 13). Additionally, fewer patients in the leflunomide group required retreatment during the observational period. No difference in the reported rate of adverse events was observed across the treatment groups.

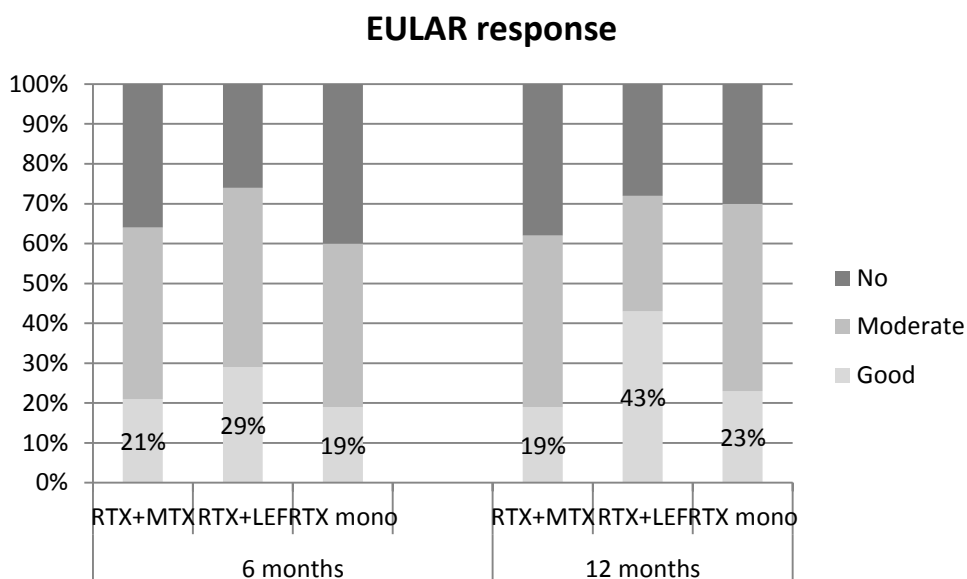


Figure 13. Effectiveness of RTX+MTX, RTX+LEF and RTX monotherapy based on EULAR response at 6 and 12 months.

4.1.3 Paper III

The total number of patients who fulfilled the inclusion criteria for this study was 2873. The vast majority (91.4%) received treatment with the approved dose rituximab, namely 1000mg x 2, while a small number of patients (248) received a lower dose of 500mg x 2. These two groups were not balanced for baseline characteristics. Patients treated with the lower dose regimen were older, had longer disease duration, a lower number of prior biologic DMARDs and lower baseline DAS28.

After adjustment for age, sex, disease duration, number of prior biologics, baseline DAS28, concomitant DMARDs and glucocorticoids, the mean DeltaDAS28 at 3 months was greater for the higher dose of rituximab, but at 6 months the difference disappeared and the mean DeltaDAS28 was similar between the two different RTX doses. Similarly, no difference was observed regarding EULAR responses and proportion of patients on remission between the groups. Effectiveness outcomes in TNFi-naïve RTX treated patients and in those who received rituximab after the failure of at least one TNFi did not differ significantly between the two treatment groups (table 7).

4.1.4 Paper IV

Analysis of repeated retreatments

777 patients received at least 4 cycles of rituximab during the observational period. Of those 81.6% were female, 83.8% were RF positive and 78.1% were anti-CCP positive. The mean \pm SD age was 55.7 ± 12.1 years and median disease duration 11 years (IQR=6-18). Patients had failed a mean of 3.0 (SD=1.6) prior synthetic DMARDs and 1.3 (SD=1.1) prior biological DMARDs. Mean baseline (=time of 1st rituximab cycle) DAS28-ESR and HAQ was 5.7 (SD=1.4) and 1.6 (SD=0.7), respectively. The majority (80.3%) of all patients received concomitant synthetic DMARD treatment, while 66% received concomitant glucocorticoids.

	anti-TNF naïve			anti-TNF failure		
	500mg x 2	1000mg x 2	p-value	500mg x 2	1000mg x 2	p-value
Baseline DAS28	5.4±1.4	5.9±1.4	0.008	6.0±1.2	6.0±1.3	NS
DAS28 3 months	4.4±1.3	4.3±1.3	NS	4.3±1.1	4.2±1.3	NS
DAS28 6 months	4.1±1.3	4.3±1.2	NS	4.3±1.4	4.1±1.3	NS
DeltaDAS28 3 months	0.9±1.3	1.6±1.5	0.08*	1.7±1.1	2.0±1.3	0.08
DeltaDAS28 6 months	1.4±1.3	1.8±1.4	NS	1.9±1.6	2.2±1.4	NS
EULAR Good Response	18.6%	14.7%	NS	19.4%	23.0%	NS
EULAR Moderate Response	62.8%	70.4%	NS	62.9%	64.7%	NS
EULAR No Response	18.6%	15.0%	NS	17.7%	12.4%	NS
Remission (DAS28<2.6)	14.0%	8.8%	NS	9.7%	13.7%	NS

Table 7. Effectiveness of two doses of RTX (500mg x 2 and 1000mg x 2) for TNFi-naïve RA patients and for those who have previously failed treatment with TNFi.

By using mixed effect models analysis we observed significant further DAS28 decline with each treatment cycle. Comparison between curves revealed significant difference between all cycles. The percentage of patients in remission and with EULAR good response was higher at the beginning of every cycle compared to the previous one. These findings suggest that for patients that continue with rituximab a further improvement in clinical assessments can be expected and support multiple treatments with rituximab.

Retreat on-flare or before a flare (fixed)?

A total of 800 patients were retreated at least 2 times and the reason for retreatment was stated: for 616 of them the reason was flare (442 at 1st and 174 at 2nd retreatment) and for 184 of them it was a fixed retreatment (128 at 1st and 56 at 2nd retreatment). Patients receiving fixed retreatment had a significantly higher (in absolute number) DeltaDAS28 ($p < 0.0001$) at the start of each cycle, compared to those retreated on-flare (figure 14). In the adjusted mixed model analysis, we compared the two retreatment groups for the 1st and the 2nd retreatment separately using estimated marginal means.

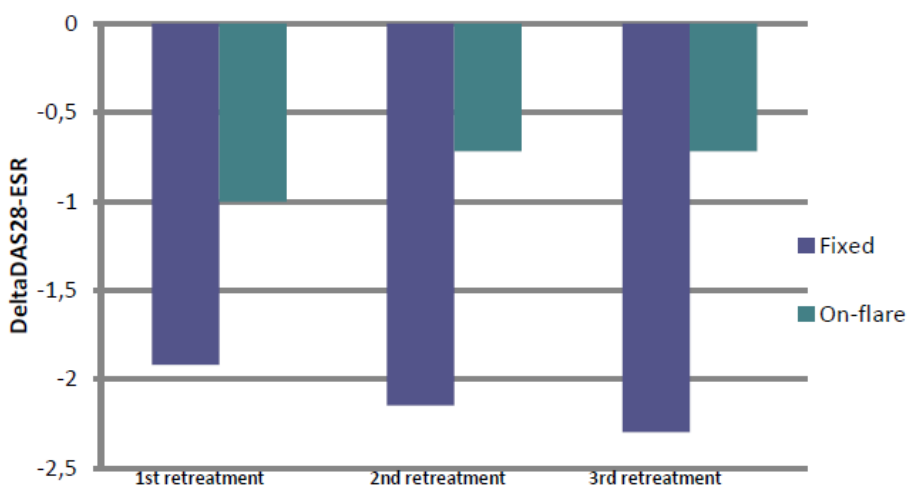


Figure 14. DeltaDAS28(ESR) at the start of each retreatment from baseline DAS28 (start of 1st cycle with RTX) for patients treated in a fixed schedule and those treated on-flare.

4.2 PAPERS V-VIII: ASPECTS OF TNF INHIBITOR USE IN RHEUMATOID ARTHRITIS: SWITCHING, CYCLING AND DISCONTINUING

4.2.1 Paper V

For this analysis we identified patients from ARTIS register who switched within 2 months to a second TNFi after the failure of a 1st TNF. After applying the inclusion criteria 952 patients were identified and included in the analysis. Regarding the effectiveness in the total groups of patients, significant reductions in DAS28 were observed for all 3 TNFis at 6 months: Δ DAS28 0–6 months = -1.1 ± 1.5 [n=38] for INF, -1.4 ± 1.6 [n=275] for ETA and -0.8 ± 1.5 [n=244] for ADA. The inter-drug difference was statistically significant between ETA and ADA ($p < 0.0001$). After adjustment for baseline DAS28, this difference remained significant ($p = 0.04$). The percentage of patients who achieved Δ DAS28 ≥ 1.2 was also significantly higher for ETA compared to ADA. When the effectiveness of switching was assessed as a function of the 1st TNFi, overall better results were observed when patients were switched from a monoclonal antibody (adalimumab or infliximab) to etanercept while worse results were observed for those switching from etanercept to adalimumab. When the reason for discontinuation of the 1st TNFi was taken into consideration, better results (rate of low disease activity/remission) at 6 months were observed with the 2nd TNFi when the reason for switch was loss of efficacy or intolerance. The best responses were observed when switching to ETA after losing efficacy of ADA or INF as the 1st TNFi (table 8).

During the first 24 months after switching to the 2nd TNFi, 567 patients (60%) discontinued their second TNFi: 46 out of 74 in the INF group (62%), 257 out of 448 (57%) in the ETA group and 264 out of 430 (61%) in the ADA group. The median (95% CI) survival time for INF, ETA and ADA was 14 (7–21), 24 (16–32) and 16 (9–23) months, respectively. Significant differences were observed in drug-survival between infliximab and the other two TNFis.

	Primary inefficacy					
	INF → ETA N=65	INF → ADA N=15	ETA → INF N=18	ETA → ADA N=115	ADA → INF N=5	ADA → ETA N=68
DAS28 baseline	5.7±1.2 [52]	5.6±0.7 [11]	5.3±1.2 [15]	5.1±1.3 [92]	4.9±2.1 [3]	5.2±1.1 [57]
DAS28 6 months	4.3±1.5 [52]	4.5±1.2 [10]	4.0±1.2 [13]	4.3±1.5 [82]	3.9±2.0 [4]	3.9±1.5 [47]
DeltaDAS28 6 months*	-1.7±1.5 [43]	-1.3±1.3 [8]	-1.1±1.3 [11]	-0.7±1.3 [71]	-0.6±0.7 [2]	-1.3±1.5 [42]
	Secondary inefficacy					
	INF → ETA N=90	INF → ADA N=42	ETA → INF N=22	ETA → ADA N=101	ADA → INF N=7	ADA → ETA N=75
DAS28 baseline	5.2±1.3 [76]	5.2±1.3 [32]	4.9±1.0 [16]	4.7±1.3 [78]	5.0±1.6 [7]	5.0±1.1 [67]
DAS28 6 months	3.5±1.5 [70]	3.3±1.4 [25]	3.2±1.7 [15]	4.0±1.5 [71]	2.9±0.3 [3]	3.7±1.5 [53]
DeltaDAS28 6 months**	-1.7±1.5 [59]	-1.3±1.6 [17]	-1.6±1.2 [11]	-0.9±1.6 [62]	-0.5±0.6 [3]	-1.4±1.6 [50]

	Intolerance					
	INF → ETA N=57	INF → ADA N=23	ETA → INF N=5	ETA → ADA N=51	ADA → INF N=2	ADA → ETA N=30
DAS28 baseline*	5.1±1.7 [47]	4.0±1.6 [18]	4.9±1.5 [5]	4.6±1.4 [38]	4.1±2.0 [2]	4.9±1.6 [23]
DAS28 6 months **	3.8±1.6 [36]	3.0±1.9 [17]	4.2 [1]	3.6±1.4 [33]	2.9±0.3 [2]	4.2±1.7 [21]
DeltaDAS28 6 months***	-1.5±1.5 [32]	-1.0±2.2 [14]	-1.5 [1]	-0.9±1.4 [27]	-1.3±1.7 [2]	-0.7±1.8 [18]
	Other					
	INF → ETA N=30	INF → ADA N=21	ETA → INF N=13	ETA → ADA N=58	ADA → INF N=2	ADA → ETA N=31
DAS28 baseline*	4.8±1.7 [22]	4.0±1.3 [17]	4.9±1.7 [9]	4.0±1.5 [46]	4.8±0.9 [2]	4.2±1.3 [27]
DAS28 6 months **	3.5±1.1 [20]	2.8±0.6 [12]	4.1±1.5 [9]	3.7±1.2 [37]	5.9 [1]	3.6±1.2 [18]
DeltaDAS28 6 months***	-1.5±1.5 [14]	-1.2±0.9 [9]	-0.7±2.4 [7]	-0.3±1.7 [34]	0.5 [1]	-0.2±1.4 [14]

Table 8. Effectiveness of switching split by type of 1st TNFi and reason for discontinuation of 1st TNFi

4.2.2 Paper VI

We identified 259 patients who switched to a 2nd TNFi and 69 who switched to RTX after the failure of a 1st TNFi. Both treatments yielded significant results during the first 6 months of therapy. The mean (SD) DAS28 improvement was significantly lower for infliximab and adalimumab (group of TNFi monoclonal antibodies) compared to RTX and etanercept. The difference remained statistically significant after adjustment for baseline differences (age, RF, baseline DAS28, HAQ). When the effectiveness of switch was examined as a function of the type of the 1st TNFi, we observed significantly greater EULAR Good response rate for RTX (36.9%) compared to mAb (11.1%) ($p= 0.001$) after the failure of etanercept. After the failure of mAb, RTX and ETA yielded similar EULAR Good/Moderate response rates (no statistical difference was observed) that were numerically higher than mAb (figure 15).

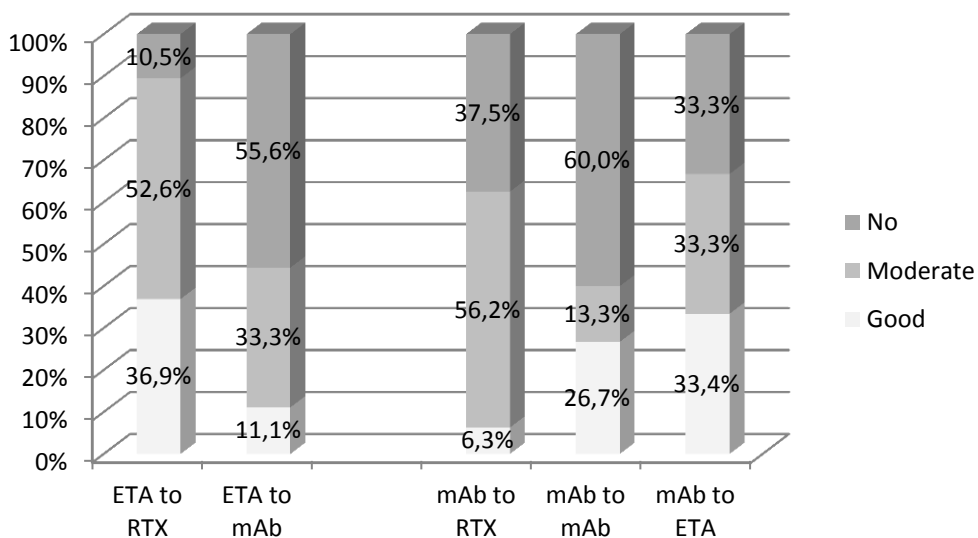


Figure 15. EULAR responses at 6 months for patients switching to RTX or a TNFi (ETA or mAb) after the failure of a 1st TNFi according to the type of the 1st TNFi.

When the cause for switching was intolerance, RTX achieved significantly greater improvement in DAS28 ($p=0.02$, 95% CI 0.08–0.89) and significantly

greater EULAR Good/Moderate response rates ($p=0.04$) compared to anti-TNF mAb. When the cause for switching was ineffectiveness, the improvement in DAS28 was similar for RTX, ETA, and mAb. The EULAR Good/Moderate responders were 60, 70, and 40.4% for RTX, ETA, and mAb, respectively ($p=0.05$ between RTX and mAb, $p=0.02$ between mAb and ETA). The number of patients however was quite small to draw any safe conclusions.

4.2.3 Paper VII

During the period 01 October 2009 – 31 June 2013, 945 patients with a diagnosis of RA who started treatment with certolizumab pegol were identified and selected. 540 patients (57.1%) received certolizumab as 1st biologic treatment, 215 patients (23%) had tested 1 previous TNFi and 190 (20%) had tested at least 2 TNFis. Patients who had failed at least 2 prior TNFis had significantly longer disease duration, higher disease activity and more functional disability at baseline compared to TNFi-naïve patients. Out of 753 patients with available DAS28 information at baseline, 292 (39%) had high disease activity ($\text{DAS28} > 5.1$) while 461 (61%) had non-high activity in their disease ($\text{DAS28} \leq 5.1$). The proportion of patients in remission at 6 months for patients with 0, 1 and ≥ 2 prior TNFis was 42%, 26% and 18%, respectively (the difference being strongly significant between all three groups, $p < 0.0001$), (figure 16). Overall 38%/33%/29% achieved EULAR good/moderate/no response at 6 months from baseline. The proportion of EULAR good responders was significantly greater for patients with 0 prior TNFis compared to those with 1 and ≥ 2 prior TNFis at 3 and 6 months. At 6 months patients with high disease activity at baseline achieved significantly greater DeltaDAS28 compared to those with non-high disease activity at baseline, but patients with non-high disease activity achieved remission to a significantly greater extent compared to those with high disease activity initially (43% vs. 21%, $p < 0.0001$). EULAR Good response rates were similar between the two groups (39% and 36%, $p=0.6$).

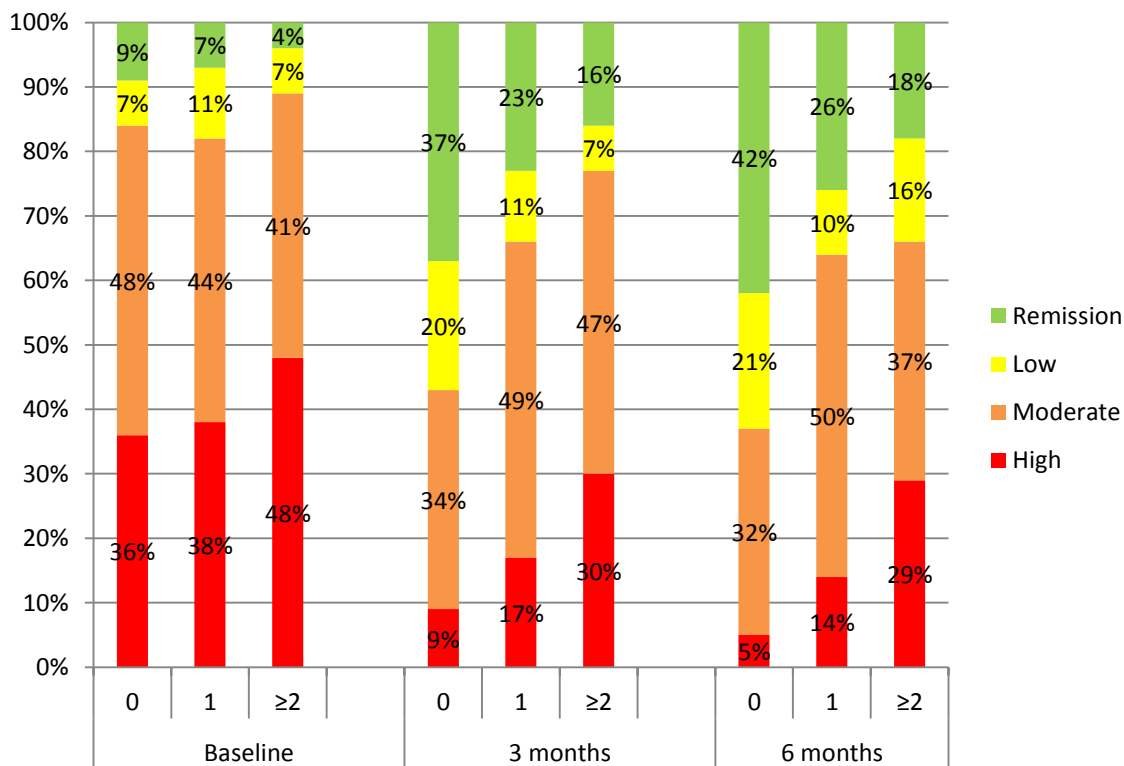


Figure 16. Proportion of patients in DAS28 disease activity state (high, moderate, low and remission) at baseline, 3 and 6 months for RA patients treated with certolizumab pegol according to the number of prior TNFis used (0, 1 or ≥2).

4.2.4 Paper VIII

A total of 237 patients with adalimumab + methotrexate were screened. Only 33 patients were eligible and included in the study, 17 were randomized to arm AM and 16 to arm M. One patient was excluded in each arm due to protocol violation.

At week 28 the vast majority of patients in AM group were still on remission (15/16) while only 1/3 of patients in M group were on remission (5/15), ($p=0.001$). The proportion of patients with at least one flare during the first 28 weeks was 50% (8/8) in AM group and 80% (12/15) in M group ($p=0.08$). Interestingly, analysis on the subgroups of flares showed significant difference in the proportion of patients with at least one $\Delta\text{DAS28} > 1.2$ but no difference in the proportion of patients with at least one $\text{DAS28} \geq 2.6$ ($p=0.2$). When different definitions of flare were tested the difference between AM and M group became clearer.

Patients who flared had longer disease duration and started treatment with ADA later than those who did not flare in both M and AM groups during the study period. There was also a tendency for lower baseline DAS28 in the non-flared patients. A total of 9 patients entered the rescue arm during the first 28 weeks. Remission was restored in 8 of them within 12 weeks (the one patient achieved again remission at the end, $\text{DAS28}=2.5$ at the observational follow-up visit). No unexpected safety signals were reported after reinstitution of ADA.

5 DISCUSSION

5.1 PAPERS I-IV

All about rituximab - Interpretation of findings

The four studies provided new information about the every-day use of rituximab in RA. In the first study the clinical effectiveness of RTX during the first 6 months after a first treatment course was confirmed in a real-life setting and was in agreement with the RCTs.

Several previous studies have indicated that RF seropositivity is a predictor of response to treatment, which is expected since patients with RA most likely to respond to treatment with RTX are those in whom B cells play a more important role in the pathogenesis of their disease. Such patients could potentially be identified by analyses of serum autoantibodies, including RF and anti-CCP. The study confirmed this observation, as significantly better results were seen after 6 months for RF-positive patients than for RF-negative patients, but also for anti-CCP-positive versus negative individuals and double-positive versus double-negative subjects. Based on figure 17, four interesting observations could be made which concluded the efficacy of rituximab with regard to RF and anti-CCP status.

- 1) Seropositive patients achieved generally significantly better results compared to seronegative patients (same trend for RF positive vs. RF negative, anti-CCP positive vs. anti-CCP negative and double seropositive vs. double seronegative). That was also confirmed in the multivariate logistic regression analysis.
- 2) The difference between autoantibody positive vs. negative was more striking at 3 months than 6 months. There are two possible explanations: the difference might indeed decline, suggesting that seropositive patients respond quicker than seronegative, but the latter group continue to improve from 3 to 6 months in a slower rate. However, one should not forget that this is an observational study and the risk for selection bias is present. There is a possibility that only those seronegative patients who show some response at 3 months will continue at 6 months (the number of observations is lower at 6 compared to 3

months) while those who do not respond adequately will ‘discontinue’ treatment. It is however common in the clinical practice to wait at least 6 months to assess the treatment effectiveness, so it is less likely that a patient will start another treatment before the 6 month visit.

- 3) Seronegative patients also showed significant improvement both at 3 and at 6 months. Immunologically this is explainable, as B cells are not only antibody producing cells, but also, as it is explained in detail in the introduction, also potent antigen-presenting cells and even responsible for cytokine production. In a seronegative population it might be these aspects of B cells that are affected. This could perhaps support the hypothesis of a ‘slower effect’ of rituximab in seronegative patients. Finally, what we call seronegative might not be really seronegative, as there can be autoantibodies that we do not screen for or even unknown autoantibodies today.
- 4) The difference seems to be more striking in the anti-CCP group between positive and negative patients compared to the RF group. This observation suggest that anti-CCP might be a stronger predictor of response to rituximab treatment compared to RF. The results of the multivariate logistic regression analysis supported this finding.

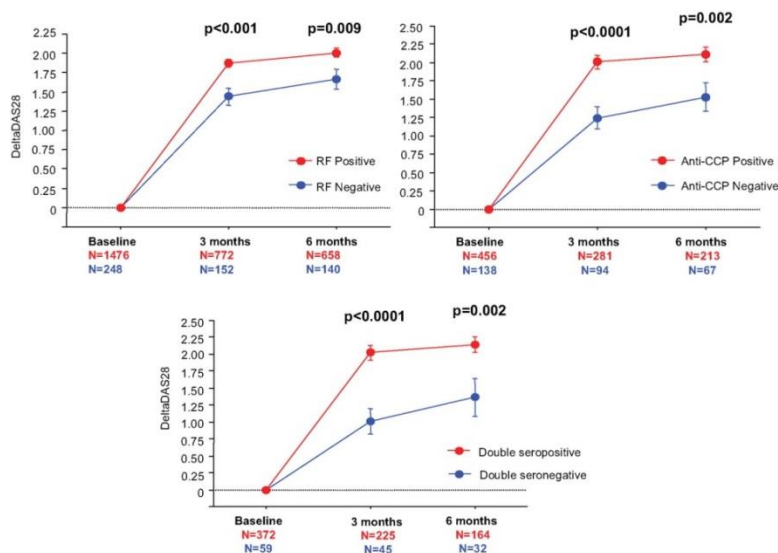


Figure 17. Mean DeltaDAS28 (bars: SEM) during the first 6 months of therapy with rituximab for seropositive and seronegative patients according to RF and anti-CCP status.

The off-label use of rituximab as first biologic DMARD, before a TNFi, appears to be highly effective in RA, as it was also shown in RCTs. In this study it was shown that the earlier rituximab is introduced the better the results, with the mean DAS28 reductions to be significantly greater in patients with 0 or 1 prior TNFis compared to those who had already failed to or more TNFis. This was independent of other possible confounders, such as anti-CCP and baseline disease activity. This observation however is not unique for rituximab. Several studies, including the study VII in this thesis, have shown that effectiveness is declining parallel with the number of biologic agents used. Risk for selection bias is present in this observation, since patients with more difficult to treat disease or intolerance are more likely to change more than two biologic agents. At the time of initiation of the second or the third biologic most of them will have a long disease activity, perhaps with high level of functional disability because of chronic damage, which can negatively affect the response to the response to the biologic agent. The main conclusion that can be drawn from this analysis is that rituximab can be used with good results in biologic naïve patients, especially those with contraindications for TNFis. I would also personally choose to treat some seropositive patients with rituximab directly after the failure of a synthetic DMARDs, an approach closer to the ‘individualized’ and ‘targeted’ treatment terms. Seropositivity and response to rituximab is after all the stronger predictor of good response to treatment with a biologic agent today.

Other studies have found similar results regarding seropositivity and number of prior biologic DMARDs used before rituximab¹⁰⁰⁻¹⁰². Results from RCTs also support the higher efficacy of rituximab in the seropositive population. In the REFLEX trial protection from joint damage was only evident in the seropositive patients⁷⁰. Other predictors of response to rituximab that we did not had the possibility to examine in the CERERRA cohort are the degree of B-cell depletion⁵⁰, the Fcγ receptor type IIIA polymorphism¹⁰³, heterozygosity for FCGR3A genotype¹⁰⁴, and several others.

Regarding the combination of rituximab with conventional DMARDs, the results of the second study suggest that leflunomide is at least as effective as methotrexate, if not better. This has important clinical implications, since a significant number of patients do not tolerate methotrexate. Leflunomide has a different safety profile than methotrexate and can be a very good alternative as monotherapy but also as a concomitant DMARD in the treatment with TNFi and rituximab. From an immunological aspect, the observation that leflunomide would lead to greater improvement in disease activity compared to methotrexate raised thoughts about a potential synergistic mechanism between leflunomide and rituximab. Indeed, there are studies from the hematology field that have shown that leflunomide has an impact on B-cell proliferation and cell cycle progression, thus suppressing antibody responses^{105,106}. On the contrary, neither methotrexate nor TNFis were able to decrease the frequency of autoreactive B-cell clones. Some other studies have supported our findings¹⁰⁷.

Another interesting observation regarding concomitant treatment with conventional DMARDs was that no significant difference was observed between the RTX+MTX and RTX monotherapy groups. A small study found earlier similar results. Almost all RCTs though assessed the efficacy of rituximab in combination with methotrexate, except for one study where rituximab monotherapy was associated with significant ACR20 and ACR50 responses compared to placebo group (methotrexate monotherapy) but not for ACR70¹⁰⁸. These results and our results suggest that when the combination with a conventional DMARD is not possible because of contraindications or intolerance rituximab can be considered as monotherapy.

Hitherto there have been no proper dose-response studies for rituximab in RA. What is considered the “autoimmune dose and protocol” was developed by Prof Jo Edwards based on a small number of patients¹⁰⁹. Our study provides some additional evidence about the lack of any striking difference and perhaps no clinically significant difference between two different doses of rituximab used in clinical practice. This finding is consistent with those from the DANCER and SERENE trials^{110,111}. In the MIRROR trial the percentage of EULAR

Good/Moderate response was borderline significantly higher in the 1000mg x 2 (89%) compared to the 500mg x 2 group (73%) ($p=0.05$)¹¹². The overall conclusions of the MIRROR trial was that the two rituximab doses could not be clearly differentiated, although some clinical outcomes were in favor of the higher dose. MTX-naïve patients in the IMAGE trial responded equally well to both doses, but only the higher dose of rituximab was proven effective in inhibiting progression of joint damage¹¹³. Despite the fact that the clinical significance of this small difference observed can be discussed, it raises an important aspect of biologic treatment, and that is the ability to inhibit radiographic progression. Unfortunately radiological data have not been collected in the CERERRA cohort and thus it was not possible to examine the ability of the two different dosing regimens to inhibit joint destruction in clinical practice. A recent meta-analysis on four RCTs did not demonstrate a significant difference in the clinical responses between the high- and low-dose rituximab regimens¹¹⁴.

A study from UK has shown that response to rituximab may be more dependent on the level of B-cell depletion, assessed by highly sensitive flow cytometry, rather than the dose. In that study complete depletion of B-cells was seen in both 500mg x 2 and 1000mg x 2 groups and it correlated better with response than the dose itself¹¹⁵. This might lead to a more individualized approach, which will be able to predict which patients could respond to a lower dose rituximab.

Another important clinical aspect of rituximab treatment is the number and time of retreatment. This remains unclear today. Our study supports the effectiveness of multiple treatment cycles. It is important to know that disease activity can further improve with each additional treatment cycle, especially for patients with partial response. The ultimate goal of RA treatment today is after all remission, so it is important to follow a 'treat to target' approach. Several studies hitherto support a fixed retreatment strategy, before a patient flares^{112,116,117}. The risk with a fixed retreatment schedule is the 'overtreatment' of a proportion of patients that can achieve long-standing response after the first treatment cycle without needing retreatment. This subgroup of patients is however quite small,

approximately 10% of all rituximab treated patients. It is important to better define this subgroup in order to differentiate it from the rest of RA patients who will need repeated retreatments with rituximab.

Strengths and limitations

Significant strengths of these studies were:

- The **large number of patients** included. The total number of RTX treated patients in the last datacut was over 4000, which makes it the largest RTX cohort today. This enables us to perform specific analyses on subsets and subgroups that would not be possible with a smaller cohort, such as the leflunomide + rituximab, the 500mg x 2 and the multiple retreatment subgroups.
- The **real-life character of the cohort**. Here I would like to introduce a certain terms, the internal validity and external validity. While randomized clinical trials are usually characterized by good internal validity, meaning the accuracy of the conclusions about the intervention's effect, the generalizability of the results to the general population can be problematic. Clinical trials have often strict inclusion and exclusion criteria, which does not reflect reality. A study showed that only a minor proportion of patients in a real-life register would have been eligible for the major clinical trials. Comorbidities, intolerance to investigational co-medications, previous treatments are some examples of exclusion criteria for clinical trials often seen in RA patients in the clinic. A major difference between patients included in clinical trials and 'real-life' RA patients is the level of disease activity at the time of initiation of a biologic agent. While most RCTs demand high disease activity as inclusion criterion, most RA patients in clinical practice have a DAS28 less than 5.1, that means non-high disease activity. As it was shown in paper I, 27% of patients did not have high disease activity when

they started rituximab. For the above reasons, register-based observational studies that examine the effectiveness of treatments have a **good external validity**, and their results are easily generalizable.

- The **international character** of the CERERRA cohort allowed the comparison of different populations, with different treatment protocols and routines. The **off-label treatment** of rituximab as first biologic was observed in more than one-third of patients. In Russia rituximab was the only biologic agent used for many years, and that influenced the results. However, around 20-30% of all patients in the other participating countries also received rituximab as first biologic agents. The main reason for that was comorbidities (tuberculosis, past or current malignancies including hematological malignancies).

Limitations of these studies include:

- The significant **heterogeneity between countries**. There were differences in laboratory methods for determining RF and anti-CCP status. Additionally there were significant differences in baseline characteristics, such as disease activity status. Some of these differences of course achieved a statistical level of significance mainly because of the large number of observations, without being clinically significant. We tried to partially overcome this problem by adjusting for country in the regression analysis. In paper IV country was included in the mixed model analysis as random variable, in order to take into consideration the variability between countries.
- The **observational and non-controlled character** of the studies introduces risk for several biases. In paper II patients treated with leflunomide had most likely previously not tolerated methotrexate or had contraindications for methotrexate, which can result in **confounding by indication** or selection bias. Confounding by indication would however most likely bias the results towards the null, as leflunomide is often

prescribed to more difficult patients. Similarly, in paper III we identified a risk for **channeling bias**, as patients treated with the lower dose were older, had higher disease duration, lower disease activity at baseline and lower number of prior biologics and were more often treated with corticosteroids and less often with concomitant DMARDs. The lower dose group may therefore represent a population of patients with more comorbidities, for whom the treating rheumatologist chose the lower dose of RTX. However, such a population would be more prone to have a worse response to therapy, and therefore confounding by indication would bias the results against the lower dose RTX. Although several analyses were adjusted for differences between groups under comparison, the risk for **residual confounding** or **confounding by unmeasured factors** was present.

- Some of the improvement in effectiveness can be explained by **regression to the mean** and **placebo effect**. This however does not differ from RCTs.
- **Missing data** is always a concern in register-based studies and an important limitation. For most of the analysis missingness was not informative, meaning that the level of missing data was the same for subgroups. This is important as one of the reason for missing information might be because of discontinuation of treatment. In contrast to other biologic agents, the definition of discontinuation for rituximab is more complex. Higher rate of missing data in a particular subgroup might indicate higher rate of discontinuation and thus worse response to treatment or even higher rate of infections.

5.2 PAPERS V-VIII

Interpretation of findings

To switch or not to switch? True inter-drug differences?

One of the main findings of the fifth study is that switching to a second TNFi may lead to significant clinical improvements. Almost 40% of patients achieved low disease activity or remission, regardless of the specific TNFi. So switching does make sense, which is very important for both treating rheumatologists and patients.

Some differences were observed between TNFis. Etanercept was associated with better results compared to adalimumab. Baseline disease activity was significantly higher for those starting etanercept as their second TNFi than adalimumab, which (under the assumption that the capacity for DAS28 reduction is non-linear, or simply through regression to the mean) might explain the greater improvement in DAS28 for etanercept as second TNFi compared with adalimumab. To exclude effect modification we stratified by baseline disease activity and we observed overall greater reductions in DAS28 among those patients with a disease activity score above 5.1 at the time point of the second TNFi start than in the non-high disease activity group, but, as expected, a higher percentage of patients achieving low disease activity/remission in the latter. Etanercept achieved numerically but not significantly better improvements than adalimumab, which might represent a true effect but also reflect limited statistical precision. The difference was not large enough to assure a true clinical difference.

Confounding by indication may be responsible for some of the previously observed differences in drug survival between ETA and ADA/INF as first TNFi. In this study, we could limit some of such confounding by indication by assessing drug survival on these drugs when used as the second TNFi (hence, when patients starting ETA were recruited from a pool of patients who had initially all be channeled to treatment with INF or ADA, and vice versa). We found some differences in favor of ETA as second TNFi, similar to what has

been shown in previous analyses of first TNFi, suggesting that it is more possible that these results represent true difference and are not entirely due to confounding by indication related to the choice of the first TNFi. The clinical relevance of the observed difference is, however, unclear. Other differences between TNFis, such as route of administration, have to be taken into account. INF is given intravenously, which might influence negatively its retention rate. Such factors limit the possibility of comparing these agents using retention rates as surrogates for effectiveness.

Does effectiveness of switching depend on the type of 1st TNFi and the reason for switching?

Overall better results were achieved with the second TNFi after loss of efficacy or intolerance to first TNFi than after lack of efficacy of the first TNFi, supporting the results from previous studies. This observation is rational and supports the hypothesis that for patients who do not respond to TNFi (primary inefficacy), TNF might not play that important role in their disease and a second TNFi would yield only modest results. When different switching strategies were compared, overall better results were observed for patients who switched from INF or ADA (monoclonal antibodies) to ETA than the other way round. The production of antidrug antibodies, drug immunogenicity, has been proposed as one possible mechanism behind inefficacy¹¹⁸⁻¹²¹. Secondary inefficacy to a first TNFi might be due to development of antidrug antibodies, and non-responders in that case might benefit from a switch to a less immunogenic drug, such as ETA. A clinical interpretation of this observation might be that after the failure of a monoclonal anti-TNF antibody one could consider switching to ETA, but when a patient has failed ETA as first TNFi, the choice of a monoclonal antibody as second TNFi might not have the same potential to lead to a clinically significant improvement. Indeed, other studies have shown that in the latter case it might be more beneficial to change to a biologic of a different mechanism of action, as it was shown in study number VI. Another mechanism that could explain this finding, could be the following: as it is known, ETA competitively inhibits the binding of both TNF and lymphotoxin- α to cell surface TNF receptors, rendering TNF biologically

inactive¹²², while INF and ADA bind and neutralize both soluble and membrane-bound TNF but not lymphotoxin. Lymphotoxin plays a crucial role in chronic inflammation¹²³. Thus, failing a monoclonal antibody (especially because of inefficacy) a patient might respond well to ETA, and the reason could be the binding of lymphotoxin¹²⁴. This remains, however, a hypothesis.

Switching from a TNFi to another TNFi versus RTX

The results of study nr VII support partially the results of the study above. In a smaller cohort (which was though part of the larger cohort based on the ARTIS register) switching to a TNFi mAb after the failure of a mAb was associated with worse results, while switching from a mAb to etanercept can yield equally good results as switching to a biologic with a different mechanism of action such as RTX. However, when patients fail etanercept switching to a mAb was associated with poor results, such as in paper V. For these patients RTX is a better alternative. A recent study from Spain concluded similar results⁷⁶.

Regarding the reason for switching, our results were not in agreement with previous study, such as the study from Finckh et al, who found better results when switching mechanism of action because of ineffectiveness⁷⁴. In our study better results with RTX compared to TNFi were observed when the reason for discontinuation of the first TNFi was intolerance or secondary ineffectiveness. We would however expect to see a difference when patients switch treatment because of primary inefficacy, as for patients who do not respond at all to a first TNFi, TNF might not play an important role, and switching biologic class is preferable. When interpretating our results, one should take into account two important things: first, there was a low power because of the limited number of patients, so certain differences could not achieve statistical significance; second, this was an observational study, and the choice of biologic in clinical practice is never random and there is a significant risk for selection bias. More patients switched from TNFi to RTX because of intolerance.

Some limitations that we have to address for the two switching studies include the observational cohort study design (absence of randomization), which can introduce the risk for selection bias, the imbalance between treatment groups regarding baseline characteristics, the limited number of patients in some of the comparisons (lack of power) and missingness.

The results could be summarized in the algorithm below.

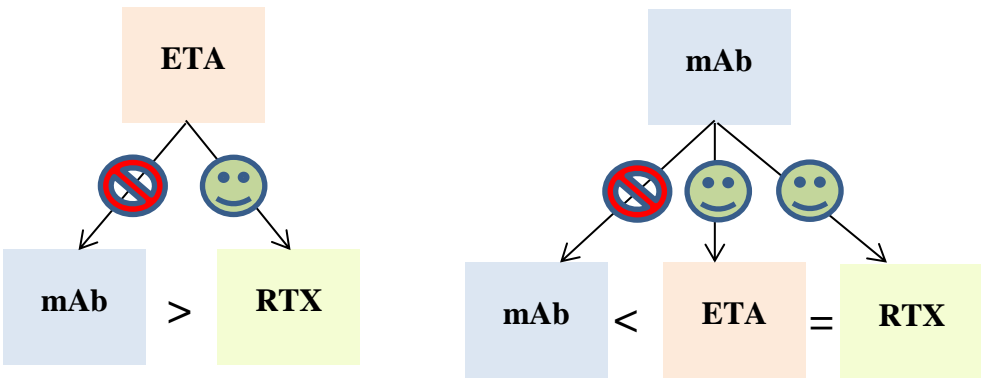


Figure 18. Algorithm about switching to TNFi or RTX after the failure of a 1st TNFi.

Certolizumab pegol: did we need one more TNFi?

In study nr VII, the effectiveness of the newer TNFi certolizumab pegol was clearly demonstrated. As shown in study nr V, cycling between TNFis can lead to significant clinical outcomes, which is now well established^{69,125,126}. In this study, patients who were naïve to TNF inhibition achieved significantly greater DAS28 reduction at 3 and 6 months from initiation of certolizumab pegol

compared to those having failed one and those having failed two or more prior TNFis. The proportion of patients in remission and who achieved EULAR good response was significantly greater for TNF naïve patients compared to both other groups. This finding was expected and is in agreement with previous findings from observational studies showing inferior response rates when switching to a second or third TNFi^{69,126}.

However, even after the failure of 2 or more TNFis 55% of patients can achieve EULAR good or moderate response and around 20% can achieve remission. These results are partially in agreement with the results from the REALISTIC study, where the efficacy of certolizumab pegol was demonstrated even for patients with previous TNFi use, regardless of the number or type of previous TNFi used¹²⁷.

In this study more than half of patients had low or moderate disease activity based on DAS28 at the time of certolizumab pegol initiation, reflecting the real-life character of the cohort. Clinically and statistically significant responses were observed regardless of whether the initial disease activity state was high or not. Patients with non-high disease activity initially were more likely to achieve remission and to continue with the treatment compared to those with high activity. These results are in agreement with the CERTAIN trial, where low-disease activity or remission was reached by the majority of patients who received certolizumab pegol and who had predominantly moderate disease activity at baseline¹²⁸. Finally, the use of concomitant DMARDs was associated with longer survival-on-drug in the Cox regression analysis. Previous studies have shown that concomitant DMARD treatment is associated with better response to treatment with TNFis¹²⁹.

The end: discontinuation of biologic treatment after remission is achieved, possible or not?

The main result of the last study suggested that patients with established RA who are in stable remission on adalimumab and methotrexate can rarely maintain remission after discontinuation of the TNFi. Discontinuation of adalimumab

might be feasible only for a small, but still considerable, fraction of patients, around 20%. Reinstitution of the biological agent was not associated with poorer results as before discontinuation or with unexpected safety signals.

The next step is trying to identify these patients. In this relatively small study there was a clear trend regarding achievement of 'biologic-free remission' and early initiation of the TNFi. So the earlier the biologic treatment is introduced from the time of RA diagnosis the higher the chance to discontinue the biologic agent and remain in remission. Several studies have suggested this association.

Thus a more aggressive treatment approach in RA might not only lead to better clinical, functional and radiographic outcomes, but it could increase the possibility to discontinue treatment without deteriorating. The latter would have vast implications both for long-term safety but also for health-economics, taking into consideration the high cost of the biologic treatment.

6 CONCLUSIONS

- i. Rituximab is effective in RA in a real-life setting. The effectiveness is higher in seropositive patients and in those who have previously failed at most one TNFi. Seronegative patients can also respond but perhaps not as quick as seropositive patients.
- ii. Leflunomide is an effective and safe alternative to methotrexate as concomitant treatment with rituximab. It can yield even better results than methotrexate, suggesting a possible synergistic mechanism. Monotherapy can also lead to significant clinical results.
- iii. While the approved dose of rituximab in RA is 1000mg x 2 with two weeks interval, the lower dose of 500mg x 2 seems to be equally effective in reducing disease activity.
- iv. Repeated rituximab cycles can lead to further DAS28 improvement. A fixed retreatment strategy yields better results than retreatment 'on-flare'.
- v. After the failure of the first TNFi, up to 40% of patients switching to a second TNFi may achieve low disease activity or remission. After the failure of a monoclonal antibody as first TNFi because of inefficacy, switching to etanercept yielded good clinical results, but not the other way round. Etanercept was associated with longer drug survival compared with infliximab as second TNFi.
- vi. After failure of etanercept as 1st TNFi, switching to rituximab yields better results compared to switching to a monoclonal antibody TNFi.
- vii. The newer TNFi certolizumab pegol is highly effective in real-life RA patients, especially for TNFi-naïve patients but even for patients who have previously failed TNF inhibition. Co-treatment with synthetic DMARDs is associated with longer survival-on-drug..
- viii. Discontinuation of TNFi may be feasible in only a minority of patients with established RA in stable clinical remission. Earlier initiation of anti-TNF treatment might increase the chance to biologic-free remission.

7 FUTURE PLANS

Many interesting questions have been raised and are going to be investigated further:

- i. Identification and characterisation of a subset of RA patients who achieve long-term response after one treatment cycle of rituximab, without need for retreatment.
- ii. What is the optimal dose of rituximab at retreatment?
- iii. Does the level of RF and anti-CCP at the time of rituximab start influence the response to treatment?

Several of the findings of the above studies could be tested further in a laboratory setting, from ‘bedside to bench’:

- i. Are there specific immunological characteristics of seronegative patients who respond well to rituximab? Are other autoantibodies present?
- ii. Does the combination of rituximab and leflunomide lead to higher degree of B-cell depletion?
- iii. Is the degree of B-cell depletion both in the periphery and in synovium similar for the two different doses of RTX?

Other findings could be examined for confirmation in a different setting, for example in a pragmatic clinical trial

- i. Assessment of the efficacy and safety of the higher and lower dose RTX, and its effect on radiographic progression, both at the first treatment cycle and on retreatment.
- ii. Assessment of different switching strategies (randomization important to exclude risk for confounding by indication). Does the presence of neutralizing anti-drug antibodies influence the result of switching?
- iii. Is the earlier introduction of a TNFi associated with greater chance to achieve biologic-free remission? Is there any difference between TNFis?

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I

Highest clinical effectiveness of rituximab in autoantibody-positive patients with rheumatoid arthritis and in those for whom no more than one previous TNF antagonist has failed: pooled data from 10 European registries

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ABSTRACT

Objective To assess the 6-month effectiveness of the first rituximab (RTX) course in rheumatoid arthritis (RA) and to identify possible predictors of response.

Method 10 European registries submitted anonymised datasets (baseline, 3- and 6-month follow-up) from patients with RA who had started RTX, and datasets were pooled and analysed. Heterogeneity between countries was analysed by analysis of variance. Predictors of response were identified by logistic regression.

Results 2019 patients were included (mean age/disease duration 53.8/12.1 years, 80.3% female, 85.6% rheumatoid factor (RF) positive and 76.8% (456/594 patients) anti-cyclic citrullinated peptide antibodies (anti-CCP) positive). For these patients an average of 2.7 disease-modifying antirheumatic drugs (DMARDs) (range 0–10) had failed, and RTX was given as the first biological agent in 36.6% of patients. There was significant heterogeneity between countries for several baseline characteristics, including the number of previous biological agents. Disease Activity Score based on 28 joint counts (DAS28) decreased from 5.8 ± 1.4 at baseline to 4.2 ± 1.4 at 6 months ($p < 0.0001$) and 22.2%/42.5% achieved European League Against Rheumatism (EULAR) good/moderate response. Larger 6-month improvement in DAS28 was observed in RF-positive and anti-CCP-positive versus seronegative patients. The following predictors of EULAR good response at 6 months were identified in a multivariate analysis: anti-CCP positivity (OR = 2.86, $p = 0.003$), number of previous DMARDs (OR = 0.84, $p = 0.06$), ≤ 1 previous biological agents (OR = 1.89, $p = 0.04$), baseline DAS28 level (OR = 0.74, $p = 0.003$).

Conclusion In this large observational cohort of patients with RA treated with RTX, seropositive patients achieved significantly greater reductions in DAS28 at 6 months than seronegative patients. Effectiveness was best when RTX was used as the first biological agent or after failure of no more than one anti-tumour necrosis factor agent.

INTRODUCTION

Rituximab (RTX) (Mabthera, Rituxan), a monoclonal antibody which selectively targets CD20-positive B cells, has been approved for the treatment of rheumatoid arthritis (RA) in many countries. With the standard approved dosage, a single course

of two 1000 mg infusions given 2 weeks apart, a majority of patients in clinical trials exhibited an ACR20 (American College of Rheumatology 20% improvement) response and a similar proportion a European League Against Rheumatism (EULAR) moderate response.¹ This beneficial effect has been demonstrated in patients naive to anti-tumour necrosis factor (anti-TNF) treatment as well as in patients for whom previous anti-TNF treatment has failed.^{1–2} Re-treatment after variable intervals of 6–12 months has been shown to be effective when a relapse occurred.^{3–4} Thus, when treatment with an anti-TNF agent has failed, RTX is now an approved and logical treatment option. Given the rapid evolution in the field of biological disease-modifying antirheumatic drugs (DMARDs), there are several treatment alternatives for patients for whom one TNF inhibitor has failed—for example, alternative TNF inhibitors, interleukin 1 and interleukin 6 inhibitors and a modulator of T cell costimulation.^{5–10} For this reason, a major challenge is to identify predictors of response, based on clinical, demographic, immunological or genetic data, for a more personalised treatment approach.¹¹

Controlled clinical trials are the major source of information on efficacy and safety of drugs under experimental conditions. However, patients included in these studies do not always represent patients in clinical practice owing to strict inclusion and exclusion criteria. Furthermore, the more complex questions that govern clinical decision-making can rarely be considered adequately using randomised trials. In such instances longitudinal observational studies may provide useful information and more reliable answers to specific questions about the use of DMARDs in clinical practice. Real-life effectiveness data on RTX in RA may be particularly relevant, taking into account questions that have been raised about optimal dosing, co-medication and efficacy in seropositive versus seronegative patients.

During the past year we have initiated a European collaboration between registries from 10 different European countries, the European Collaborative Registries for the Evaluation of Rituximab in rheumatoid arthritis (CEREREA) initiative. The aim of

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this study is to present baseline characteristics of patients with RA treated with RTX, analyse 6-month effectiveness and identify possible predictors of response.

PATIENTS AND METHODS

The CERERRA is an investigator-led, industry-supported initiative aiming to evaluate clinical aspects of RTX use in patients with RA. This manuscript was prepared from the authors without any influence by the supporting medical industry. All 10 participating European registries (from the Czech Republic, Denmark, Finland, The Netherlands, Norway, Russia, Slovenia, Spain, Sweden and Switzerland) submitted fully anonymised datasets with baseline characteristics, including age, gender, disease duration, number of previous synthetic and biological DMARDs, rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibodies (anti-CCP) status of all patients with an established diagnosis of RA who had been treated with RTX.^{12–20} Levels of RF and anti-CCP were determined by local laboratories and local cut-off values for positivity were applied. Disease activity markers at baseline and after 3 and 6 months were also provided (number of swollen and tender joints, Visual Analogue Scales for pain, patient's and physician's global assessment, Disease Activity Score based on 28 joint counts (DAS28), Health Assessment Questionnaire) as well as information about concomitant drugs (DMARDs and glucocorticoids).

Statistical analysis

Appropriate parametric statistical tests were used for the analysis of data. All continuous data were examined for normal distribution by Kolmogorov–Smirnov test. Heterogeneity for baseline characteristics between countries was analysed by analysis of variance followed by post hoc test (Bonferroni/Dunn). Clinical response according to reductions in DAS28 and achievement of EULAR response criteria was analysed at 3 and 6 months. A second analysis was performed according to the

number of previously used biological agents. Patients re-treated with RTX before 6 months were classified in the analyses as 'non-responders', regardless of their response according to the EULAR criteria.

Predictors of EULAR good response were identified by logistic regression analyses. We performed univariate logistic regression analyses adjusted for age and sex with EULAR good response at 6 months as the dependent variables and several demographic and disease variables considered to be clinically important as independent variables. The results from these analyses ($p < 0.25$ as the criterion) and correlation analyses (Pearson and Spearman correlations) guided the selection of variables for the multivariate logistic regression analyses with dichotomised responses as the dependent variables. Age and sex were also included in the multivariate analyses. The non-significant variables were removed by stepwise backward selection. We then added back into the models, one at a time, any variable not originally selected from the univariate analyses. The variables were kept in the models if significant. Appropriate tests for linearity, interactions and goodness of fit were performed. Statistical analyses were done with StatView 5.0.1 for PC (SAS Institute, Cary, North Carolina, USA) and SPSS 15.0 for Windows.

RESULTS

Baseline characteristics

The baseline characteristics for all patients are summarised in table 1 and shown according to country in online supplementary table S1. A total of 2019 patients were included with a mean (SD) age/disease duration of 53.8 (13.3)/12.1 (8.9) years. There was significant heterogeneity between the countries for age and disease duration. Patients in the Russian registry had the lowest mean age and shortest disease duration.

RF positivity was reported in 85.6% of the patients (table 1). Of the 594 patients with available data on anti-CCP status, 76.8%

Table 1 Baseline characteristics for all patients from the 10 European registries (Russia (Ru), Sweden (Swe), Norway (Nor), Finland (Fin), Denmark (Den), Slovenia (Slo), Spain (Sp), The Netherlands (Nth), the Czech Republic (Cz), Switzerland (Switz))

Baseline characteristics of all patients (N=2019)	Number of patients with available information	Mean (SD) or %	Test for heterogeneity between countries	Significant difference between countries (post hoc analyses)
			p Value (ANOVA/ χ^2)	
Age (years)	1989	53.8 (13.3)	<0.0001	Swe higher vs all other countries. Ru lower vs all other countries
Gender (% female)	1993	80.3	<0.0001	Ru higher vs Den, NL. NL higher vs Cz
Disease duration (years)	1888	12.1 (8.9)	<0.0001	Ru shorter vs Nor, Swe, Switz, Fin. Fin longer vs all countries. Swe longer vs Den
N previous DMARDs	1525	2.7 (1.6)	<0.0001	Switz and Ru lower vs Slo, Nor, NL, Den, Cz
N previous biological agents	1844	1.1 (1.1)	<0.0001	Ru lower vs all countries. Cz lower vs Den, Fin, NL, Nor
RF (% positive)	1724	85.6	<0.0001	Cz lower vs all countries. Switz higher vs Cz, Den, Nor, Ru and NL
Anti-CCP (% positive)	594	76.8	0.01	Fin highest percentage, Den lowest
DAS28	1730	5.8 (1.4)	<0.0001	Between almost all countries. Nor highest, Fin lowest.
HAQ	843	1.5 (0.7)	0.002	Cz higher vs Fin, Switz
Concomitant corticosteroids (%)	1824	60.9	<0.0001	Cz and Den lower vs Fin, NL, Nor, Ru, Slo, Sp, Swe, Switz. Ru higher vs Slo, Sp, Swe, Switz
Concomitant DMARDs (%)	1920	76.7	<0.0001	NL lower vs all countries. Switz higher than Swe, Ru, Den

ANOVA, analysis of variance; anti-CCP, anti-cyclic citrullinated peptide antibodies; DAS28, 28-joint count Disease Activity Score; DMARDs, disease-modifying antirheumatic drugs; HAQ, Health Assessment Questionnaire; RF, rheumatoid factor.

were positive. The numbers of confirmed double seropositive and double seronegative patients were 372 and 59, respectively. RF-positive patients were significantly older than RF-negative patients (mean (SD) age 54.9 (13.0) vs 50.3 (14.5), $p<0.0001$). No difference was found between the RF-positive and RF-negative subgroups for the number of previous DMARDs and biological agents, baseline DAS28, disease duration or concomitant DMARDs. Anti-CCP-positive patients had a significantly smaller number of previous biological DMARDs than anti-CCP-negative patients, but other differences in baseline characteristics were not observed between anti-CCP-positive and anti-CCP-negative patients.

The mean (SD) numbers of previous synthetic and biological DMARDs were 2.7 (1.6) and 1.1 (1.1), respectively. Patients in the Russian registry had used a significantly smaller number of previous biological DMARDs than patients from the other registries (online supplementary table S1).

Information about previous use of biological DMARDs was available in 1844 patients. For these patients two or more biological agents had previously failed in 596 (32.3%), one agent had failed in 574 (31.1%), and 674 (36.6%) patients received RTX as their first biological agent. The baseline characteristics stratified by number of previous biological agents are summarised in online supplementary table S2.

Concomitant synthetic DMARDs were used by 76.7% of the patients. The majority were co-treated with methotrexate (MTX) (64.8%), a small number received combination treatment with MTX and another synthetic DMARD, while a significant number of patients were treated with other DMARDs,

such as sulfasalazine, azathioprine, ciclosporin, leflunomide, antimalarial agents and also gold salts in a few patients.

Treatment responses

The mean (SD) DAS28 at baseline was 5.8 (1.4) and decreased to 4.3 (1.3) and 4.2 (1.4) at 3- and 6-month assessments, respectively. The majority of patients (73.0%) had high baseline disease activity (DAS28 >5.1) and 21.8% had moderate disease activity (DAS28 between 3.2 and 5.1). At 3 months the percentage of patients with high disease activity was markedly reduced and remained stable at the 6-month assessment (figure 1). A corresponding increase in the proportions of patients with low disease activity or in remission was seen (figure 1). EULAR good/moderate responses at 3 months were achieved by 195/483 out of 1087 patients (17.9%/44.4%) and by 210/402 out of 945 patients (22.2%/42.5%) after 6 months. DAS28 improvement of >1.2 was observed in 62.5% of patients with available Δ DAS28 at the 6-month follow-up (616/986 patients).

The subset of RF-positive patients achieved significantly larger reductions in DAS28 than the patients who were RF negative (95% CI of the difference at 3 months -0.64 to -0.18 , at 6 months -0.64 to -0.09) (table 2 and figure 2). Similar results were seen when anti-CCP positive and anti-CCP negative, as well as when double-positive versus double-negative patients were compared (table 2 and figure 2). The proportions of patients with EULAR good/moderate response are also depicted in table 2.

The majority of patients received a concomitant DMARD. At 3 months, responses were similar in patients with and without synthetic DMARD co-treatment, but after 6 months the mean improvement of DAS28 was significantly larger for patients receiving concomitant DMARDs (Δ DAS28 1.9 (1.5) vs 1.6 (1.5), $p=0.04$, 95% CI for the difference -0.47 to -0.03).

The mean (SD) improvement in DAS28 at 6 months for patients with no or one previous biological treatment was 2.2 (1.4) and 2.0 (1.4), respectively, while for patients for whom two or more biological agents had failed, the improvement was 1.3 (1.6) (figure 3). The difference between the first two groups and the third group was statistically significant ($p<0.0001$ at 3 and 6 months). At 6 months, the percentage of patients who achieved EULAR good/moderate response was 23.1%/50.6%, 20.5%/46.6% and 20.2%/29.8% for patients with no, one or more than one previous biological agents, respectively.

Predictors of response

Possible predictors of EULAR good response at 6 months were identified by univariate logistic regression analysis adjusted for

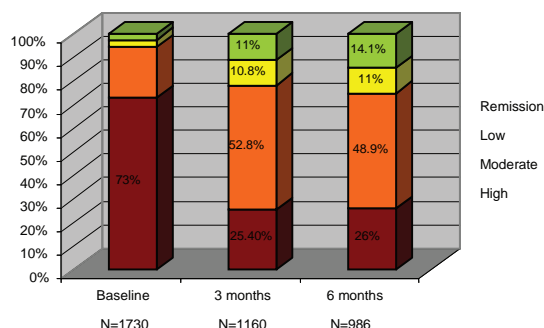


Figure 1 Levels of DAS28 at baseline, after 3 and 6 months.

Table 2 Reductions in DAS28 at 3 and 6 months (mean (SD)) and achievement of EULAR good/moderate response (%) by RF and anti-CCP status

	Positive	Negative	p Value	Positive	Negative	p Value
	DAS28 improvement at 3 months (N=1073)			DAS28 improvement at 6 months (N=930)		
RF	1.8 (1.3)	1.4 (1.3)	0.0005	1.9 (1.5)	1.6 (1.5)	0.009
Anti-CCP	1.9 (1.4)	1.1 (1.3)	<0.0001	2.0 (1.5)	1.4 (1.6)	0.002
Double	1.9 (1.4)	0.9 (1.1)	<0.0001	2.1 (1.5)	1.1 (1.6)	0.001
	EULAR good/moderate response at 3 months (N=1087)			EULAR good/moderate response at 6 months (N=945)		
RF	17.7/45.9%	15.0/39.2%	0.10	23.3/42.9%	20/37.9%	0.17
Anti-CCP	20.2/47.9%	6.4/39.4%	<0.0001	25.7/42.5%	14.5/39.1%	0.05
Double	20.4/47.8%	4.4/37.8%	0.001	25.5/43.6%	15.2/36.4%	0.13

N = total number of patients for whom data on DAS28 improvement/EULAR response at 3 and 6 months were available. anti-CCP, anti-cyclic citrullinated peptide antibodies; DAS28, 28-joint count Disease Activity Score; EULAR, European League Against Rheumatism; RF, rheumatoid factor.

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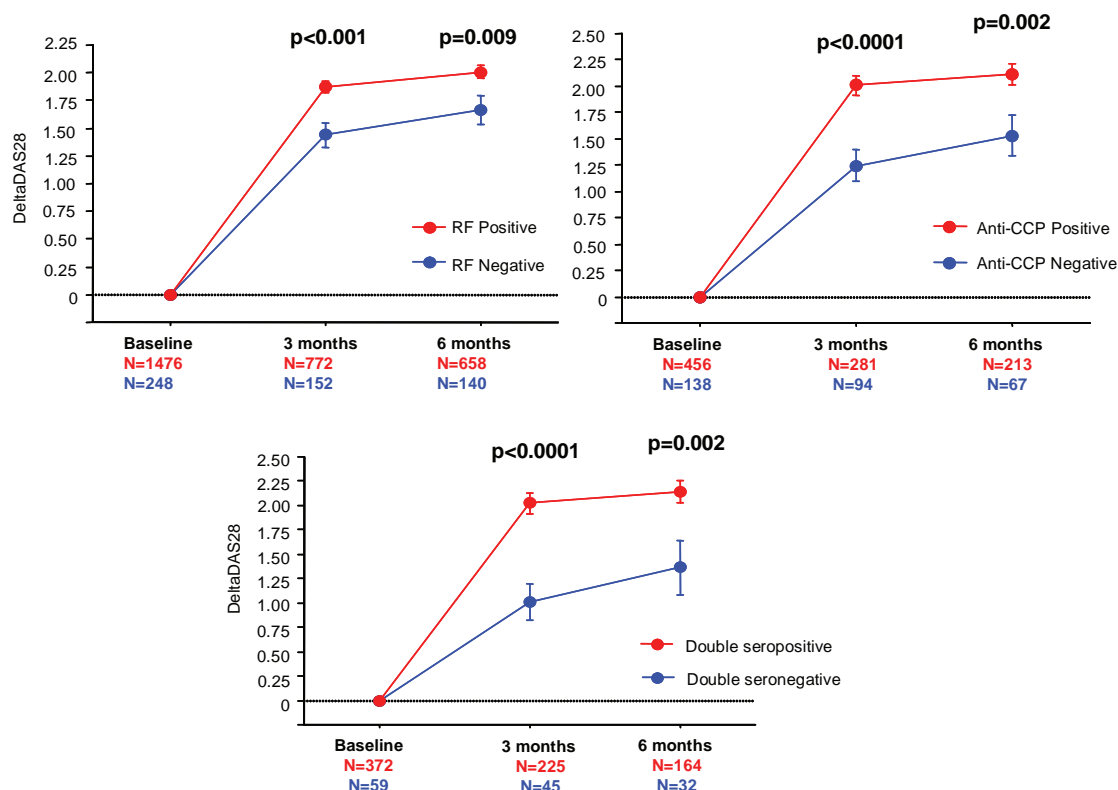


Figure 2 Mean Δ DAS28 (bars: SEM) during the first 6 months for seropositive and seronegative patients. At 3 and 6 months RF positive patients (A) as well as anti-CCP positive (B) and double positive patients (C) achieved significantly larger reductions of DAS28 compared to RF negative, anti-CCP negative and double negative patients, respectively.

age and sex (table 3). In the subsequent multivariate analysis previous use of ≤ 1 versus > 1 biological DMARDs, lower baseline DAS28 level and anti-CCP positivity were significant predictors of EULAR good response, while a smaller number of previous synthetic DMARDs was borderline significant (table 3). Since only a fraction of patients had available anti-CCP status, we performed a second multivariate analysis without anti-CCP and obtained the same results, but with somewhat stronger levels of significance (data not shown).

DISCUSSION

In this large observational cohort study, the majority of patients treated with RTX had longstanding RA and several previous synthetic DMARDs and/or at least one anti-TNF had failed as in several randomised clinical trials with RTX.¹⁻² In addition, there was a significant off-label usage of RTX in anti-TNF naive patients.²¹⁻²² The heterogeneous population with data from 10 countries allowed us to evaluate the real-life effectiveness of RTX and stratification according to number of previous biological agents and seropositivity led to relevant conclusions.

Clinical effectiveness of RTX during the first 6 months after a treatment course was confirmed in this observational study. Previous studies have indicated that RF seropositivity is a predictor of response to treatment, which is expected since patients with RA most likely to respond to treatment with RTX are those in whom B cells play a more important role in the pathogenesis

of their disease. Such patients could potentially be identified by analyses of serum autoantibodies, including RF and anti-CCP.¹⁻²³⁻²⁴ The study confirms this observation, as significantly better results were seen after 6 months for RF-positive patients than for RF-negative patients, but also for anti-CCP-positive versus negative individuals and double-positive versus double-negative subjects. However, seronegative patients also responded well to RTX. The difference between seropositive and seronegative patients at 6 months was not as strong as at 3 months. Moreover, DAS28 reduction from 3 to 6 months did not differ between the two groups. One possible explanation is that seronegative patients respond more slowly than patients who are seropositive. While initial reports suggested a lack of effectiveness of RTX in seronegative patients,⁴⁻²⁵ more recent studies have suggested that RTX can be effective for such patients as well.¹⁻² These results could be explained by the observation that B cells play an important role in the pathogenesis of RA as autoantibody producers and also as antigen-presenting cells and proinflammatory cytokine-releasing cells. It is also theoretically possible that seronegative responders produce autoantibodies not as yet known. There is, however, a risk of underlying bias: only seronegative patients with a satisfactory response continue to receive RTX for 6 months, and therefore the patients for whom we have 6 months' data could be selected from the initial population. To recommend use of RTX in seronegative patients further support from large observational or clinical studies is needed.

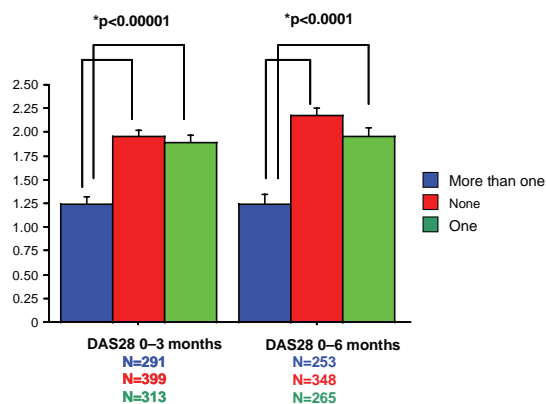


Figure 3 Mean DAS28 improvement (bars: SEM) for patients who failed none, one or more than one biologic DMARD. The mean reductions in DAS28 during the first 3 and 6 months were significantly greater for patients having failed at most one biologic compared to those who had two or more prior biologics ($p<0.0001$).

Table 3 EULAR good response: univariate and multivariate logistic regression analyses

Variable	Coefficient	OR (95% CI)	p Value
Univariate adjusted analyses*			
Previous synthetic DMARDs	-0.17	0.84 (0.75 to 0.94)	0.002
Previous biological DMARDs 0-1 (vs ≥ 2)	0.55	1.73 (1.25 to 2.38)	0.001
HAQ at baseline	0.18	1.20 (0.80 to 1.70)	0.30
Disease duration	-0.01	0.99 (0.98 to 1.01)	0.48
Rheumatoid factor positive (vs negative)	0.44	1.50 (1.01 to 2.36)	0.04
DAS28 at baseline	-0.08	0.90 (0.87 to 1.02)	0.11
Concomitant synthetic DMARD yes (vs no)	-0.05	0.95 (0.67 to 1.33)	0.75
Concomitant glucocorticoids yes (vs no)	0.26	0.77 (0.57 to 1.04)	0.09
Anti-CCP positive (vs negative)	1.09	3.00 (1.56 to 5.77)	0.001
Multivariate adjusted analysis*			
Anti-CCP positive (vs negative)	1.05	2.86 (1.43 to 5.71)	0.003
Previous biological DMARDs 0-1 (vs ≥ 2)	0.64	1.89 (1.02 to 3.51)	0.04
DAS28 at baseline	-0.29	0.74 (0.61 to 0.91)	0.003
Previous synthetic DMARDs	-0.18	0.84 (0.7 to 1.01)	0.06

*Adjusted for age and sex.

anti-CCP, anti-cyclic citrullinated peptide antibodies; DAS28, 28-joint count Disease Activity Score; DMARDs, disease-modifying antirheumatic drugs; EULAR, European League Against Rheumatism; HAQ, Health Assessment Questionnaire.

Anti-CCP was found in the univariate and multivariate analysis to be a significant independent prognostic marker of EULAR good response after 6 months, with an OR of 2.86 (95% CI 1.43 to 5.71). RF positivity was shown to be a significant predictor of EULAR good response in the univariate analyses, but did not remain significant in the multivariate model, even though the patients numbers with information about RF was much higher than the number with information about anti-CCP. These observations may indicate that anti-CCP positivity is a stronger predictor of response to RTX than RF seropositivity. Longer follow-up, larger patient numbers

and prospective data collection will allow us to reach more robust conclusions about seropositivity and response to RTX in the future.

In this study a large proportion of patients (36.6%) received RTX before having received an anti-TNF. RTX has more recently been tested in randomised controlled clinical trials of inadequate responders to synthetic DMARDs.^{21 22} Future examination of the specific reasons for use of RTX as first biological agent might be of value. Patients naive to anti-TNF agents and other biological agents, as well as patients for whom only one previous biological agent has failed seemed to respond significantly better to treatment than patients for whom two or more biological agents had failed. This observation suggests that earlier initiation of RTX might lead to better results. Alternatively, lack of response to two or more anti-TNF agents might suggest resistance to treatment. The latter suggestion was supported by the lack of association between delay to RTX treatment (baseline disease duration) and response.

Observational studies have indicated that RTX might be a better alternative than switching between TNF antagonists after a failure of anti-TNF treatment.^{26 27} We had no data available to examine this question in this analysis, but this research question may be addressed later in the CERRERA database.

Another interesting result was a slight decrease in effectiveness of treatment between 3 and 6 months for patients not being co-treated with DMARDs. Thus, concomitant DMARDs seem to maintain the therapeutic effect of RTX. In future analyses the effect of different DMARDs in combination with RTX will be examined.

This study started with 2019 patients. However, the number of patients analysed at 3 and 6 months was lower (figure 1). To exclude an important bias, we compared the characteristics of patients missing at these time points with those used for the analysis, which was particularly relevant for evaluation of the responses of seronegative patients with RA. As shown in figure 2B, no difference in the number of anti-CCP-positive and anti-CCP-negative patients with available data at 3 and 6 months could be detected (61.6% vs 68.1% and 46.7% vs 48.6%, respectively). Contrarily, significantly more RF-negative patients than RF-positive patients had available DAS28 at 3 (61.3% vs 52.3%) and 6 months (56.5% vs 44.6%) (figure 2A). The last observation could support our finding that seronegative patients can also respond well to RTX but more slowly than those who are seropositive. On the other hand, one could suggest that the missing data of a large amount of seropositive patients, especially at 6 months, might be related to a very good response to treatment, thus introducing a selection bias. The absence of difference in the anti-CCP subgroup allowed us to reach more robust conclusions on anti-CCP positivity as a predictor of response to RTX.

This study has some limitations, such as the significant heterogeneity between countries for baseline characteristics, differences in laboratory methods for determining RF and anti-CCP antibodies and the difficulty of splitting the countries into groups in order to include them as a covariate in the multivariate analysis. Another weakness is that the study was observational and uncontrolled. Perhaps some of the improvements can be explained by regression to the mean or the placebo effect. Also, we are using observed data and we cannot report whether patients who dropped out before follow-up visits or had missing data also had worse results. The quite high number of missing data is of concern, but it is something we expected, as it is a large observational cohort. On the other hand, the large number of patients, the fact that there are 10 participating countries with

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different treatment protocols, the 'off-label' treatment of anti-TNF naïve patients with RTX, the possibility of examining the combination of RTX with other DMARDs apart from MTX, and the fact that all data came from 'real-life' patients are significant strengths of this study.

In conclusion in this large observational cohort, RF-positive and anti-CCP-positive patients achieved significantly greater reductions in DAS28 at 6 months than seronegative patients. Anti-CCP positivity was an independent predictor of EULAR good response. Effectiveness results were best when RTX was used as the first biological agent or in patients for whom at most one anti-TNF had failed. Concomitant DMARDs may prolong the initial effectiveness, leading to longer relapse-free disease.

Ethics approval This study was conducted with the approval of the registry of each country.

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Highest clinical effectiveness of rituximab in autoantibody-positive patients with rheumatoid arthritis and in those for whom no more than one previous TNF antagonist has failed: pooled data from 10 European registries

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II

CONCISE REPORT

Effectiveness of disease-modifying antirheumatic drug co-therapy with methotrexate and leflunomide in rituximab-treated rheumatoid arthritis patients: results of a 1-year follow-up study from the CERERRA collaboration

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► Additional supplementary data are published online only. To view these files please visit the journal online (<http://ard.bmj.com/content/71/3.toc>)

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ABSTRACT

Objectives To compare the effectiveness and safety of rituximab alone or in combination with either methotrexate or leflunomide.

Methods 10 European registries submitted anonymised datasets with baseline, 3, 6, 9 and 12-month clinical data from patients who started rituximab.

Results 1195 patients were treated with rituximab plus methotrexate, 177 with rituximab plus leflunomide and 505 with rituximab alone. Significantly more patients achieved a European League Against Rheumatism good response at 6 months when treated with rituximab plus leflunomide (29.1%) compared with rituximab plus methotrexate (21.1%) and rituximab alone (19.3%; $p=0.02$ and $p=0.01$, respectively). Similar results were observed at 12 months. Adverse events occurred in 10.2%, 13.2% and 13.9% of patients on rituximab plus leflunomide, rituximab plus methotrexate and rituximab alone, respectively.

Conclusions Leflunomide is an effective and safe alternative to methotrexate as concomitant treatment with rituximab. Slightly better results were obtained by the combination of rituximab and leflunomide than rituximab and methotrexate, raising the possibility of a synergistic effect of leflunomide and rituximab.

Rituximab (Mabthera, Rituxan) has been approved for the treatment of rheumatoid arthritis (RA). Randomised controlled clinical trials with rituximab have shown significant clinical improvements for patients who failed tumour necrosis factor (TNF) inhibition and biological agent naive patients.^{1–3} In all trials rituximab was administered in combination with methotrexate.

Methotrexate and leflunomide are synthetic disease-modifying antirheumatic drugs (DMARD) that inhibit purine and pyrimidine synthesis, respectively. Both drugs have been proved effective for the treatment of RA,^{4–7} but the exact pharmacological mechanisms of action are still unclear. Leflunomide is often used in clinical practice as an alternative DMARD, either alone or in combination with biological agents for patients intolerant to

methotrexate, but limited data on the efficacy and safety of combination treatment with leflunomide and anti-TNF or other biological agents from clinical or epidemiological studies are available.⁸

Controlled clinical trials are the major source of information on the efficacy and safety of medications. However, since the inclusion of patients in these studies is governed by strict inclusion and exclusion criteria, the study populations can differ from real-life patients in several respects. We know from experience that a significant number of patients do not tolerate methotrexate, but the vast majority of clinical trials conducted to assess the efficacy of rituximab used methotrexate as anchor therapy. Furthermore, rituximab is sometimes used alone in the case of patients intolerant to several synthetic DMARD. There is therefore a need for evidence of the effectiveness and safety of rituximab either alone or in combination with synthetic DMARD other than methotrexate.

PATIENTS AND METHODS

The European Collaborative Registries for the Evaluation of Rituximab in Rheumatoid Arthritis (CERERRA) is an investigator-led, industry-supported initiative with the aim of evaluating the clinical aspects of rituximab use in patients with RA (K Chatzidionysiou, E Lie, E Nasonov, *et al*, unpublished observation). All 10 participating European registries submitted fully anonymised datasets with baseline characteristics of all RA patients who had been treated with rituximab. Levels of rheumatoid factor and anti-cyclic citrullinated peptide (CCP) were determined by local laboratories and local cut-off values for positivity were applied. Disease activity markers and disability score at baseline and after 3, 6, 9 and 12 months were also provided (see supplementary data, available online only).

Patients were separated into three groups: rituximab plus methotrexate, rituximab plus leflunomide and rituximab alone. A small number of patients was excluded (see supplementary data, available online only).

Statistical analysis

Overall differences between the groups for baseline values were tested with analysis of variance (ANOVA). For those variables that ANOVA showed to have significant heterogeneity pairwise comparison was performed with the two-tailed Student's *t* test (for normally distributed continuous data) and the χ^2 test (for categorical variables). The univariate and multivariate logistic regression analyses are described elsewhere (see supplementary data, available online only).

RESULTS

Among the 2265 RA patients who started treatment with rituximab, 1195 were treated with rituximab plus methotrexate, 177 with rituximab plus leflunomide and 505 with rituximab alone. The baseline characteristics of the patients in the three treatment groups are shown in table 1. The majority of patients were treated with 2×1000 mg rituximab according to recommendations: 91.5% of patients in the rituximab plus methotrexate, 91.4% in the rituximab plus leflunomide and 86.4% in the rituximab monotherapy group (the difference being significant only between rituximab plus methotrexate and rituximab monotherapy, *p*=0.003). The rest of the patients received 2×500 mg rituximab. The mean dose (\pm SD) of methotrexate was 14.4 \pm 5.4 mg a week. The majority of patients on rituximab plus leflunomide (95.1%) were treated with 20 mg leflunomide a day (mean \pm SD daily dosage 17.6 \pm 3.6 mg).

Patients on rituximab plus leflunomide achieved a greater reduction in disease activity score in 28 joints (DAS28) values from baseline to the 6 and 12-month assessments compared with rituximab plus methotrexate and rituximab alone (figure 1A). At 6 months the mean \pm SD Δ DAS28 from baseline in

the rituximab plus leflunomide, rituximab plus methotrexate and rituximab-alone groups were 2.1 \pm 1.3, 1.9 \pm 1.5 and 1.7 \pm 1.5, respectively (*p*=0.02 for rituximab plus leflunomide vs rituximab alone). At 12 months Δ DAS28 from baseline in the rituximab plus leflunomide, rituximab plus methotrexate and rituximab-alone groups were 2.2 \pm 1.6, 1.8 \pm 1.5 and 1.7 \pm 1.3, respectively (*p*=0.06 for rituximab plus leflunomide vs rituximab plus methotrexate and *p*=0.05 for rituximab plus leflunomide vs rituximab alone). The mean \pm SD health assessment questionnaire (HAQ) from baseline in the rituximab plus leflunomide, rituximab plus methotrexate and rituximab-alone groups were 0.5 \pm 0.7, 0.5 \pm 0.6 and 0.4 \pm 0.6, respectively, at 6 months, and 0.5 \pm 0.6, 0.4 \pm 0.6 and 0.3 \pm 0.6 at 12 months, respectively. No significant differences were observed in the HAQ reductions at 6 and 12 months between the treatment groups.

Significantly more patients achieved a European League Against Rheumatism (EULAR) good response at 6 months when treated with rituximab plus leflunomide (29.1%) compared with rituximab plus methotrexate (21.1%) and rituximab alone (19.3%), *p*=0.02 and *p*=0.01, respectively (figure 1B). At 12 months an even higher percentage of good responders was observed in the rituximab plus leflunomide group (42.6%), while the percentage of good responders remained stable in the rituximab plus methotrexate and rituximab-alone groups (*p*=0.001 and *p*=0.08 respectively; figure 1B). The number of good responders was significantly higher in patients treated with rituximab alone than in the rituximab plus methotrexate group (*p*=0.04).

The combination rituximab plus leflunomide was significantly associated with a good EULAR response compared with rituximab plus methotrexate and rituximab monotherapy in a

Table 1 Baseline characteristics of rituximab-treated patients who received concomitant treatment with methotrexate, leflunomide or no DMARD (rituximab monotherapy)

Baseline characteristics of patients	Rituximab plus methotrexate (N=1195)	Rituximab plus leflunomide (N=177)	Rituximab monotherapy (N=505)	ANOVA (overall differences)	Pairwise comparisons (t test/ χ^2)
Age, years (mean \pm SD)	51.9 \pm 13.1	52.3 \pm 12.1	55.2 \pm 12.9	<i>p</i> <0.0001	Methotrexate plus rituximab vs rituximab <i>p</i> <0.0001, Leflunomide plus rituximab vs rituximab <i>p</i> =0.001
N	1188	177	500		
Gender (% female)	81.3	83.1	81.1	NS	NS
N	1194	177	503		
Disease duration, years (mean \pm SD)	11.7 \pm 8.8	11.4 \pm 7.9	13.2 \pm 10.1	<i>p</i> =0.007	Methotrexate plus rituximab vs rituximab <i>p</i> =0.003, Leflunomide plus rituximab vs rituximab <i>p</i> =0.04
N	1161	169	465		
No of previous DMARD (mean \pm SD)	2.6 \pm 1.5	2.5 \pm 1.4	2.8 \pm 1.8	<i>p</i> =0.008	Methotrexate plus rituximab vs rituximab <i>p</i> =0.003, Leflunomide plus rituximab vs rituximab <i>p</i> =0.05
N	1019	162	400		
No of previous biological agents (mean \pm SD)	0.9 \pm 0.8	0.6 \pm 0.8	1.0 \pm 0.8	<i>p</i> <0.0001	Methotrexate plus rituximab vs rituximab <i>p</i> =0.01, Leflunomide plus rituximab vs rituximab <i>p</i> <0.0001, Methotrexate plus rituximab vs Leflunomide plus rituximab <i>p</i> =0.001
N	1136	170	464		
RF (% positive)	74.5	77.6	78.8	NS	NS
N	1044	147	415		
Anti-CCP (% positive)	73.6	76.1	76.9	NS	NS
N	444	71	143		
DAS28 (mean \pm SD)	5.9 \pm 1.3	5.9 \pm 1.2	5.7 \pm 1.3	<i>p</i> =0.029	Methotrexate plus rituximab vs rituximab <i>p</i> =0.02
N	1108	155	433		
HAQ (mean \pm SD)	1.6 \pm 0.7	1.6 \pm 0.7	1.7 \pm 0.7	NS	NS
N	850	137	323		
Use of glucocorticoids (%)	59.9	53.2	56.6	NS	NS
N	1175	171	442		
Glucocorticoids dose (mg)	8.9 \pm 7.9	7.1 \pm 5.0	9.9 \pm 10.4	<i>p</i> =0.008	Methotrexate plus rituximab vs Leflunomide plus rituximab <i>p</i> =0.02, Leflunomide plus rituximab vs rituximab <i>p</i> =0.01
N	696	99	251		

N=number of patients in each group with available data.

ANOVA, analysis of variance; CCP, cyclic citrullinated peptide; DAS28, disease activity score in 28 joints; DMARD, disease-modifying antirheumatic drug; HAQ, health assessment questionnaire; RF, rheumatoid factor.

Clinical and epidemiological research

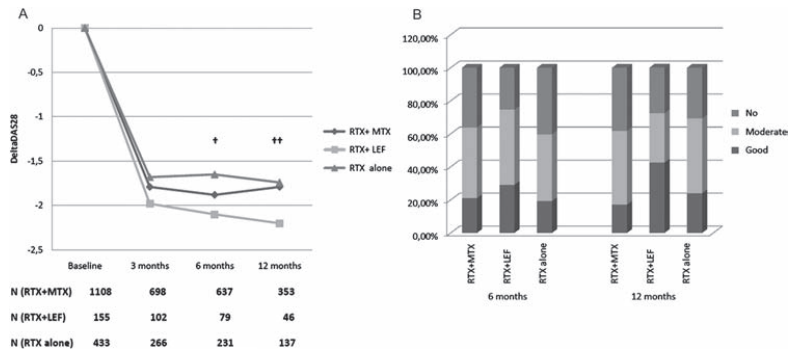


Figure 1 (A) Efficacy of different concomitant treatments with rituximab at 6 and 12 months after first treatment according to the disease activity score in 28 joints (DAS28) reductions from baseline to 12 months. Patients treated with rituximab plus leflunomide achieved numerically greater DAS28 reductions at 6 and 12 months compared with rituximab plus methotrexate and rituximab-alone-treated patients, with the differences being statistically significant between rituximab plus leflunomide and rituximab alone at 6 ($p=0.02$) and 12 months ($p=0.05$), and borderline significant between rituximab plus leflunomide and rituximab plus methotrexate at 12 months ($p=0.06$). The numbers of patients in each treatment group with available Δ DAS28 at each time point are shown. $\dagger p=0.02$ rituximab plus leflunomide versus rituximab alone. $\ddagger p=0.06$ rituximab plus leflunomide versus rituximab plus methotrexate and $p=0.05$ rituximab plus leflunomide versus rituximab alone. (B) Effect of different concomitant treatments with rituximab at 6 and 12 months after first treatment according to the European League Against Rheumatism (EULAR) response (good, moderate or none). Significantly more patients achieved a EULAR good response at 6 months when treated with rituximab plus leflunomide (29.1%) compared with rituximab plus methotrexate (21.1%) and rituximab alone (19.3%), $p=0.02$ and $p=0.01$, respectively. At 12 months an even higher percentage of good responders was observed in the rituximab plus leflunomide group (42.6%), while the percentage of good responders remained stable in the rituximab plus methotrexate (17.1%) and rituximab-alone groups (24%) ($p=0.001$ and $p=0.08$, respectively). LEF, leflunomide; MTX, methotrexate; RTX, rituximab.

Table 2 Results of the separate univariate analyses for each variable, adjusted for age and sex, at 6 and 12 months

	Variables	6 Months		12 Months	
		OR (95% CI)	p Value	OR (95% CI)	p Value
Univariate analysis*	Disease duration	1.0 (0.9 to 1.0)	0.28	1.0 (0.7 to 1.5)	0.82
	RF (positive vs negative)	1.4 (0.9 to 2.0)	0.1	1.5 (1.1 to 2.2)	0.02
	Anti-CCP (positive vs negative)	2.5 (1.4 to 4.5)	0.002	2.5 (1.4 to 4.4)	0.001
	Previous DMARD (n)	0.8 (0.7 to 0.9)	0.001	1.0 (0.9 to 1.1)	0.36
	Previous biological agents (n) (0–1 vs >1)	1.8 (1.3 to 2.5)	<0.0001	1.6 (1.2 to 2.2)	0.004
	Baseline DAS28	0.8 (0.5 to 1.1)	0.17	1.0 (0.9 to 1.1)	0.96
	Baseline HAQ	0.9 (0.8 to 1.2)	0.7	0.9 (0.6 to 1.0)	0.15
	Leflunomide plus rituximab vs methotrexate plus rituximab	2.1 (1.2 to 3.5)	0.009	2.0 (1.2 to 3.3)	0.005
	Leflunomide plus rituximab vs rituximab	2.3 (1.2 to 4.1)	0.008	2.0 (1.2 to 3.5)	0.01
	Methotrexate plus rituximab vs rituximab	1.1 (0.8 to 1.6)	0.6	1.0 (0.7 to 1.5)	0.93
	Use of glucocorticoids	1.3 (1.0 to 1.8)	0.06	1.3 (1.0 to 1.8)	0.04
	Glucocorticoids dose	0.9 (0.9 to 1.0)	0.45	0.9 (0.9 to 1.0)	0.02
	Country†		0.001		0.002
Multivariate analysis‡	Previous biological agents (n) (0–1 vs >1)	1.5 (0.9 to 2.3)	0.05	NS	NS
	Leflunomide plus rituximab	2.0 (1.1 to 3.7)	0.03	2.8 (1.0 to 7.9)	0.04
	Anti-CCP (positive vs negative)	NS	NS	2.2 (1.1 to 4.2)	0.02

*The dependent variable was EULAR good response at 6 and 12 months, respectively. The combination of leflunomide with rituximab was significantly associated with a good EULAR response compared with the combination of rituximab and methotrexate and rituximab monotherapy. Methotrexate was not associated with good response to therapy compared with rituximab monotherapy.

†Country was a significant independent variable (but no country on its own was significantly associated with good response to therapy).

‡Multivariate analysis was adjusted for age, sex and country. Concomitant treatment with leflunomide remained significant at both 6 and 12 months.

CCP, cyclic citrullinated peptide; DAS28, disease activity score in 28 joints; DMARD, disease-modifying antirheumatic drug; EULAR, European League Against Rheumatism; HAQ, health assessment questionnaire; RF, rheumatoid factor.

univariate regression analysis at 6 and 12 months adjusted for age and sex. In the multivariate analysis model adjusted for age, sex and country, a lower number of previous biological agents and concomitant leflunomide remained predictive at 6 months (table 2). At 12 months concomitant leflunomide and positive anti-CCP were significant predictors of EULAR good response to rituximab treatment (table 2).

Fewer patients with rituximab plus leflunomide required retreatment during the first 12 months (21.5%) compared with rituximab plus methotrexate (31.9%) or rituximab monotherapy (27.9%), but these differences were not statistically significant.

Adverse events occurred in 10.2%, 13.2% and 13.9% of patients in rituximab plus leflunomide, rituximab plus methotrexate and rituximab monotherapy, respectively. No difference

in the infection rate was observed (6.2% for rituximab plus leflunomide, 6.6% for rituximab plus methotrexate and 7.9% for rituximab alone). One death was reported due to aspiration pneumonia in the rituximab plus methotrexate group. In the same group, one patient was diagnosed with prostate cancer 3 months after the initiation of rituximab and one patient had an acute myocardial infarction after 6 months. In the rituximab-alone group, one acute myocardial infarction and one haemorrhagic stroke were reported. A stroke was also reported in the rituximab plus leflunomide group, 6 months after the start of rituximab treatment.

DISCUSSION

The results of this large observational cohort of RA patients support the findings of previous smaller studies reporting good results with the combination of rituximab and leflunomide.^{9 10} At both 6 and 12 months a significantly greater number of patients treated with rituximab plus leflunomide achieved a EULAR good response than patients treated with rituximab plus methotrexate and rituximab alone, and fewer patients in the rituximab plus leflunomide group required retreatment during the first year. The latter observation may suggest a longer duration of response to therapy, but this difference was not statistically significant and the criteria for retreatment were not defined according to any specific protocol. However, a consensus had been published guiding physicians about how rituximab could be used according to available evidence and expert opinion.¹¹ Co-medication with leflunomide did not lead to more adverse events. These findings thus indicate that leflunomide is a good alternative to methotrexate when used in combination with rituximab in RA.

Studies have shown that leflunomide has an impact on B-cell proliferation and cell cycle progression, thus suppressing B-cell antibody responses.^{12 13} These findings may provide a rationale regarding the mechanism of action of leflunomide in RA with a possible synergistic effect when used in combination with rituximab. Of note, methotrexate and/or TNF antagonists were not able to decrease the frequency of autoreactive B-cell clones, indicating that these anti-inflammatory therapies are not able to correct the presence of defective B-cell tolerance.¹⁴

We did not observe any difference in efficacy between patients treated with rituximab plus methotrexate or rituximab alone. Owczarczyk *et al*¹⁵ came to a similar conclusion in a small study with 40 patients.

The strengths of our study are the inclusion of a large number of patients from 10 participating countries and the 'real-life' character of the study, which provided the possibility to examine the combination of rituximab with other DMARD apart from methotrexate. This analysis has potential limitations inherent to the analysis of observational data. Almost all of the patients treated with leflunomide had either potential contraindications to methotrexate treatment or had previously not tolerated methotrexate, which could result in selection bias or confounding by indication. However, confounding by indication would most likely bias the results towards the null, as leflunomide is usually prescribed to more difficult patients with either problems of tolerance or inadequate response to methotrexate. While we could adjust our analysis for many important disease characteristics, we cannot exclude the possibility of residual confounding or confounding by unmeasured factors. Missing data are another concern with observational studies. Nevertheless, the proportion of patients at these time points with available effectiveness data was similar for the rituximab plus methotrexate group

(57.5% and 31.9%) and the rituximab plus leflunomide group (51% and 29.7%) at 6 and 12 months, respectively. The absence of radiological data was also a limitation of this study. The mean dose of methotrexate in the rituximab plus methotrexate group was relatively low (14.4 mg/week). Higher doses could lead to better results, but they are not always well tolerated.

Concomitant treatment with leflunomide remained significant in the multivariate analysis, which was adjusted for country, as well as for age, sex and the number of previous biological agents, thus allowing us to draw more robust conclusions. The weaknesses of observational studies have to be put in the perspective that no optimal randomised controlled trial has been performed to address the effectiveness and safety of co-therapy with synthetic DMARD apart from methotrexate in rituximab-treated patients. In addition, this study provides important information on patients intolerant to various DMARD who can be treated with rituximab monotherapy.

Contributors All the authors have participated in this work. KC performed the statistical analysis. KC and CG performed the study design. All authors have contributed to the drafting of the manuscript.

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Ethics approval The study was approved by different ethics committees in the different countries involved.

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Effectiveness of disease-modifying antirheumatic drug co-therapy with methotrexate and leflunomide in rituximab-treated rheumatoid arthritis patients: results of a 1-year follow-up study from the CERERRA collaboration

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III

Effectiveness of two different doses of rituximab for the treatment of RA: data from the CERERRA collaboration

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ABSTRACT

Background: The approved dose of rituximab (RTX) in rheumatoid arthritis is 1000 mg x 2, but some data have suggested similar clinical efficacy with 500 mg x 2. The purpose of this study was to compare the effectiveness of the regular and low doses given as first treatment course.

Methods: Twelve European registries participating in the CERERRA collaboration submitted anonymized datasets with demographic, efficacy and treatment data for patients who had started RTX. Treatment effectiveness was assessed by DAS28 reductions and EULAR responses after 6 months.

Results: Data on RTX dose were available for 2,873 patients, of whom 2,625 (91.4%) and 248 (8.6%) received 1000 mg x 2 and 500 mg x 2, respectively. Patients treated with 500 mg x 2 were significantly older, had longer disease duration, higher number of prior DMARDs, but lower number of prior biologics and lower baseline DAS28 than those treated with 1000 mg x 2. Fewer patients in the low-dose group received concomitant DMARDs but more frequently received concomitant corticosteroids.

Both doses led to significant clinical improvements at 6 months. DAS28 reductions at 6 months were comparable in the 2 dose regimens [mean Δ DAS28 \pm SD -2.0 \pm 1.3 (high dose) vs. -1.7 \pm 1.4 (low dose), $p=0.23$ adjusted for baseline differences]. Similar percentages of patients achieved EULAR good response in the two dose groups, 18.4% vs. 17.3%, respectively ($p=0.36$).

Conclusions: In this large observational cohort initial treatment with RTX at 500 mg x 2 and 1000 mg x 2 led to comparable clinical outcomes at 6 months.

Keywords: rituximab, rheumatoid arthritis, dose

INTRODUCTION

Rituximab (MabThera, Rituxan) is a chimeric, monoclonal anti-CD20 antibody approved for the treatment of rheumatoid arthritis (RA) in combination with methotrexate in patients with active RA who have failed at least one tumor necrosis factor (TNF) inhibitor. The efficacy and acceptable safety profile of rituximab (RTX) have been demonstrated in randomized controlled trials^{1,2} and in large observational cohorts^{3,4}. The approved dose is 1000 mg twice (with a 2-week interval) per treatment course.

There is however evidence suggesting that a lower dose of RTX, 500 mg twice, is also effective, although not approved. In the SERENE trial both 500 mg x 2 and 1000 mg x 2 RTX significantly improved clinical outcomes (ACR responses, EULAR responses, DAS28 and HAQ improvement) compared to placebo in a biologic naïve RA population⁵. The MIRROR, DANCER and IMAGE trials yielded similar results⁶⁻⁸. In all the above trials no significant difference could be detected between the different doses regarding almost all clinical outcomes⁵⁻⁸.

The purpose of this study was to assess and compare the effectiveness at 6 months between the higher (1000 mg x 2) and lower (500 mg x 2) dose of RTX given as first treatment course in a merged dataset from observational cohorts.

METHODS

The CERERRA (The European Collaborative Registries for the Evaluation of Rituximab in Rheumatoid Arthritis) is an investigator-led initiative aiming to evaluate clinical aspects of RTX use in patients with RA^{3,9}. Twelve participating European registries (from the Czech Republic, Denmark, Finland, The Netherlands, Norway, Portugal, Romania, Russia, Slovenia, Spain, Sweden and Switzerland) submitted fully anonymized datasets with baseline demographic and disease characteristics, including age, gender, disease duration, number of previous synthetic and biological DMARDs, rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibodies (anti-CCP) status of all patients with an established diagnosis of RA who started treatment with RTX. Disease activity markers at baseline and after 3 and 6 months were also provided (number of swollen and tender joints, Visual Analogue Scales (VAS) for pain, patient's and physician's global assessment, Disease Activity Score based on 28

joint counts and erythrocyte sedimentation rate (ESR), (DAS28-ESR), Health Assessment Questionnaire (HAQ)). Information about RA treatment, such as doses of rituximab and use of concomitant DMARDs and glucocorticoids was also included in the dataset. Effectiveness of rituximab in the higher (1000mg x 2) and the lower dose (500mg x 2) was assessed by DAS28 and HAQ status at 3 and 6 months, by the improvement of DAS28 and HAQ at 3 and 6 months, by disease activity at 3 and 6 months based on DAS28 status and by EULAR responses at 6 months.

A small number of patients were treated with other than the above doses (e.g. 750 mg) or did not provide information on the RTX dose and were therefore not included in the analysis.

Statistical analysis

Baseline characteristics of the two groups were analysed by means of descriptive statistics. For normally distributed variables mean \pm standard deviation (SD) and independent samples t-test was used, while median (interquartile range (IQR)) and Mann-Whitney U-test were used for the non-normally distributed variables. Chi-square test was used for comparison of categorical data.

Changes in DAS28 and HAQ were first compared in unadjusted analyses by independent samples t-test. Comparative adjusted analyses with correction for baseline group differences were subsequently performed by analyses of covariance (ANCOVA). In the ANCOVA analysis we adjusted for baseline variables found to differ significantly between the two groups (age, disease duration, number of prior biologics, baseline DAS28, concomitant DMARD) and for those thought to be clinically significant (concomitant corticosteroids). The number of prior DMARDs although significantly different between groups was not included in the ANCOVA because of the small number of patients with available information in the 500mg group and because of the high correlation with the number of prior biologics. Multivariate logistic regression analysis with EULAR response as dependent variable (a first analysis with EULAR good vs. moderate/no and a second one with EULAR good/moderate vs. no) and RTX dose (500 vs. 1000 mg) as well as several baseline variables as explanatory variables was performed [age, gender, RA disease duration, previous biologics, baseline DAS28, anti-CCP status, concomitant DMARDs and

corticosteroids]. Country was included in the model in an additional analysis. All statistical tests were evaluated at the 0.05 significance level. P-values and 95% confidence intervals are presented. The statistical analysis was performed with IBM SPSS Statistics version 20.

RESULTS

The total number of patients included in the cohort was 3,266, and 2,873 patients (88%) were eligible for the analyses. The large majority of patients [n=2,625, 91.4%] received 1000 mg x 2 (higher dose), and 248 patients (8.6%) received 500 mg x 2 (lower dose). The demographics and baseline disease characteristics for the two treatment groups are shown in Table 1. Patients who were treated with the lower RTX dose were older, had longer disease duration, fewer prior biologic agents but more prior DMARDs and lower baseline DAS28 than those treated with the higher dose. Additionally, fewer patients in the low-dose group received concomitant DMARDs but more frequently received concomitant corticosteroids. Baseline characteristics for those patients with available DAS28-ESR at 6 months are also shown in table 1. No significant differences between the two populations (all patients at baseline and patients with available response data at 6 months) were observed, so the missingness was not informative.

In the unadjusted analysis, the mean DAS28 improvement was greater for patients treated with the higher dose than for those treated with the lower dose at 3 months [1.9 ± 1.4 (N=991) vs. 1.3 ± 1.3 (N=125), $p < 0.0001$] and it remained significant in the ANCOVA analysis ($p = 0.004$) (table 2). The difference in mean DAS28 improvement was significant also at 6 months (2.0 ± 1.3 (N=1344) vs. 1.7 ± 1.4 (N=100), $p = 0.02$) in the unadjusted analysis. However, difference disappeared after adjustment ($p = 0.23$). Inclusion of country as a random variable in the ANCOVA analysis did not change the results. Improvements in function as assessed by HAQ were also similar between the groups both at 3 and 6 months (table 2). The proportion of patients with high, moderate and low disease activity and remission based on DAS28 was similar in the two groups at baseline, 3 and 6 months (Figure 1a), and so was the proportion of EULAR good, moderate and non-responders at 6 months (Figure 1b).

Multivariate logistic regression analysis was performed in order to examine the possible association of RTX dose with EULAR good (vs. moderate/no) and EULAR

good/moderate (vs. no) response, with adjustment for possible confounders, such as age, gender, RA disease duration, previous biologics, baseline DAS28, anti-CCP status, concomitant DMARDs and corticosteroids. RTX dose (lower dose vs. higher dose) was not a statistically significant predictor of achieving EULAR good response (OR: 1.08, 95% CI: 0.40-2.94, $p=0.88$) or EULAR good/moderate response (OR: 1.22, 95% CI: 0.37-4.09, $p=0.74$). When country was introduced in the model similar results were observed.

DISCUSSION

In this study based on data from the CERERRA collaboration RTX provided significant clinical improvement at 3 and 6 months in patients with active RA. The comparison between the higher (1000 mg x 2) and lower dose (500 mg x 2) of RTX showed significant difference for DAS28 improvement at 3 months but no significant difference on clinical effectiveness, as assessed by change in DAS28 and HAQ score at 6 months, after adjusting for baseline characteristics. EULAR response rates and remission rates were also similar between groups. The results of our study are thus consistent with those from the SERENE, IMAGE and MIRROR trials^{5,6, 8}. In the MIRROR trial the percentage of EULAR Good/Moderate response was borderline significantly higher in the 1000mg x 2 (89%) compared to the 500mg x 2 group (73%) ($p=0.05$). The overall conclusions of the MIRROR trial was that the two rituximab doses could not be clearly differentiated, although some clinical outcomes were in favor of the higher dose⁶.

Hitherto there have been no proper dose-response studies for rituximab in RA. What is considered the “autoimmune dose and protocol” was developed by Prof Jo Edwards based on a small number of patients¹⁰. Our study provides some additional evidence about the lack of any striking difference and perhaps no clinically significant difference between two different doses of rituximab used in clinical practice. Recently interesting data from the UK Leeds group showed that response to rituximab may be more dependent on the B-cells depletion rather than the dose. Although, in the small number of patients studied “incomplete” peripheral blood depletion was more often seen in the patients treated with the lower dose, “complete” depletion was seen in both groups and correlated better with response than dose itself¹¹.

There are several limitations that should be addressed: the observational character of the study, the different size of the two treatment groups under comparison (only 248 patients treated with the lower dose), and the fact that the two groups compared were not balanced for all baseline characteristics. Hence, there is a risk for channeling bias, as patients treated with the lower dose were older, had higher disease duration, lower disease activity at baseline and lower number of prior biologics and were more often treated with corticosteroids and less often with concomitant DMARDs. The lower dose group may represent a population of patients with more comorbidities, for whom the treating rheumatologist chose the lower dose of RTX. However, such a population would be more prone to have a worse response to therapy, and therefore confounding by indication would bias the results against the lower dose RTX.

The lack of radiological data is an additional limitation of the study. The ‘golden triad’ of current treatment guidelines in RA is remission (or low disease activity when remission is not possible), preservation of functional ability, and prevention of structural damage. Tak et al. showed in the IMAGE study that the 1000 mg x 2 RTX, but not the 500 mg x 2 dose, significantly inhibited progression of joint damage during the first 6 months, but inhibition of structural progression was similar from 6 months onwards^{8, 12}. However, the IMAGE trial included MTX-naïve patients of whom the majority had early RA, and its population was thus different from the population of our study. It would be interesting to further evaluate the ability of the lower RTX dose to prevent radiological progression in an established RA population that is more consistent with the routine use of RTX.

The length of response was not examined in the present study. The risk that the lower dose might be associated with shorter response cannot be ruled out and should be assessed in future studies.

The large number of patients included in the cohort, which made the comparison of the different doses of RTX possible, and the real-life character of the study are important strengths of the study.

CONCLUSIONS

In this large observational cohort initial treatment with RTX at 500 mg x 2 and 1000 mg x 2 led to comparable clinical outcomes after 6 months. This result may have some important cost implications in the treatment of patients with RA.

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	All patients			Patients with available response data at 6 months*		
	RTX 500mg x 2 N=248	RTX 1000mg x 2 N=2625	p-value (t-test, chi-square test)	RTX 500mg x 2 N=109	RTX 1000mg x 2 N=1385	p-value (t-test, chi-square test)
Sex,% female	83.9% [248]	80.3% [2625]	0.17	88.1% [109]	81.2% [1125]	0.08
Age, years	55.2±15.8 [247]	52.6±12.6 [2615]	0.002	55.5±15.1 [109]	51.1±11.8 [1380]	<0.0001
Disease duration, years	10.5 (5-18) [240]	8.1 (5-14) [2360]	0.02	10 (5-16.8) [108]	7.5 (5.1-12) [1329]	0.02
RF, % positive	81.7% [241]	81.2% [2031]	0.84	83% [106]	81.5% [876]	0.7
Anti-CCP, % positive	71.3% [101]	73.4% [806]	0.64	68% [50]	74.9% [363]	0.29
Number of prior biologics	0 (0-1) [207]	1 (0-2) [2560]	<0.0001	0 (0-1) [102]	1 (0-1) [1371]	0.003
Anti-TNF-naïve (%)	58% [207]	37.5% [2560]	<0.0001	58.8% [102]	37.1% [1371]	<0.0001
Number of prior DMARDs	2.6±1.3 [126]	2.4±1.4 [2248]	0.04	2.7±1.3 [55]	2.3±1.1 [1256]	0.02
Baseline DAS28- ESR	5.7±1.3 [215]	6.1±1.3 [2069]	<0.0001	5.9±1.3 [100]	6.3±1.2 [1344]	0.002
Baseline HAQ score	1.6±0.7 [212]	1.7±0.7 [1584]	0.48	1.6±0.7 [100]	1.8±0.7 [695]	0.001
Concomitant medication:						
– Any DMARD	72.6% [248]	83.1% [2625]	<0.0001	75.2% [109]	87.5% [1385]	<0.0001
MTX	46.4% [248]	63.4% [2625]	<0.0001	50.5% [109]	75.1% [1385]	<0.0001
Glucocorticoids	65.7% [248]	59.3% [2221]	0.06	71.6% [109]	64.7% [983]	0.15

Table 1. Baseline demographics, disease and treatment characteristics of patients treated with rituximab 500 mg x 2 or 1000 mg x 2 for all patients in the cohort at baseline and for those with available response data (DAS28-ESR) at 6 months.

[RF=rheumatoid factor, anti-CCP= anti-cyclic citrullinated peptide antibodies, DMARDs=disease modifying antirheumatic drugs, DAS28-ESR=disease activity score based on 28 joints and ESR, HAQ=health assessment questionnaire, MTX=methotrexate]. The number of patients with available information for each variable is included in brackets.

	RTX 500 mg x 2	RTX 1000 mg x 2	Unadjusted p-values (ANOVA)	Adjusted p- values**
DAS28 baseline	5.7±1.3 [215]	6.1±1.3 [2069]	<0.0001	
DAS28 3m	4.4±1.2 [138]	4.2±1.3 [1046]	0.15	
DAS28 6m	4.3±1.3 [109]	4.3±1.2 [1385]	0.99	
DeltaDAS28 3m	-1.3±1.3 [125]	-1.9±1.4 [991]	<0.0001	0.004
DeltaDAS28 6m	-1.7±1.4 [100]	-2.0±1.3 [1344]	0.02	0.23
HAQ baseline	1.6±0.7 [212]	1.6±0.7 [1584]	0.48	
HAQ 3m	1.3±0.7 [127]	1.3±0.7 [957]	0.83	
HAQ 6m	1.2±0.7 [109]	1.3±0.7 [912]	0.21	
DeltaHAQ 3m	-0.3±0.5 [115]	-0.5±0.6 [859]	0.02	0.10
DeltaHAQ 6m	-0.4±0.6 [103]	-0.5±0.7 [826]	0.13	0.27

Table 2. Effectiveness of treatment across the two treatment groups as assessed by DAS28 and HAQ status and reductions at 3 and 6 months. Crude and adjusted p-valued are presented.

** Analysis of covariance (ANCOVA) adjusted for age, sex, disease duration, number of prior biologics, baseline DAS28, concomitant DMARDs and glucocorticoids.

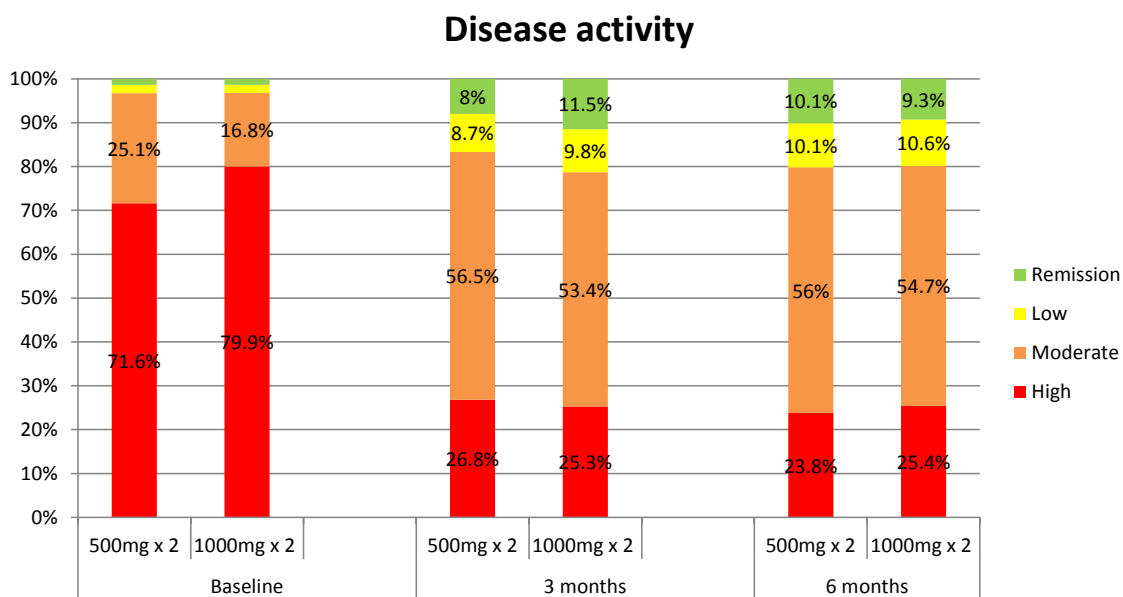


Figure 1A. Disease activity based on DAS28-ESR at baseline, 3 and 6 months in the two treatment groups (RTX 500mg x 2 and RTX 1000mg x 2). No significant differences were observed.

(Remission: $\text{DAS28} < 2.6$, Low disease activity: $2.6 \leq \text{DAS28} \leq 3.2$, Moderate disease activity: $3.2 < \text{DAS28} \leq 5.1$, High disease activity: $\text{DAS28} > 5.1$).

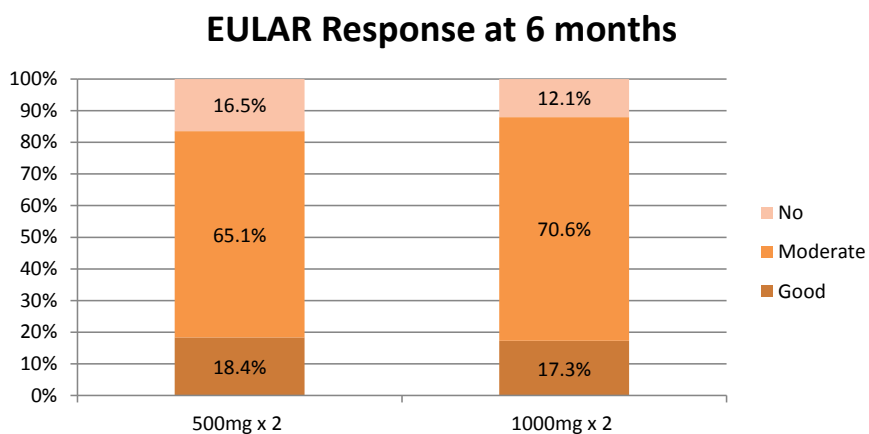


Figure 1B. EULAR Good, Moderate and No response at 6 months for the two treatment groups (RTX 500mg x 2 and RTX 1000mg x 2). No significant differences were observed.

IV

Retreatment with rituximab in Rheumatoid Arthritis in a real life cohort – data from the CERERRA collaboration

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Abstract

Background: Retreatment with rituximab (RTX) is common clinical practice, although several aspects regarding retreatment need to be further elucidated. The aim of this study was to describe the effectiveness of repeated courses of RTX and to compare two retreatment strategies, fixed retreatment and on-flare, in a large observational cohort of real-life RA patients.

Methods: Pooled data from the Collaborating European Registries for Rituximab in RA (CERERRA) cohort were used. In a first analysis, patients with RA who received at least 4 cycles with RTX were identified and included in the analysis. In a second analysis, patients who received at least 2 RTX retreatments and for whom information about the strategy for retreatment was available (according to the physician's opinion) were identified. The two retreatment strategies were compared by fitting an adjusted mixed effects model analysis to the longitudinal DAS28.

Results: 777 patients met the eligibility criteria for the first analysis. Significant improvement in DAS28 ($p < 0.001$) was observed for each course of RTX. Comparison between curves revealed significant difference between all treatment cycles.

A total of 800 patients were retreated at least 2 times: 616 retreated because of a flare and 184 at a fixed interval. Patients receiving fixed retreatment had a significantly higher (in absolute number) DeltaDAS28 ($p < 0.0001$) at the start of each cycle, compared to those retreated on-flare. In the adjusted mixed model analysis, we compared the two retreatment groups for the 1st and the 2nd retreatment separately using estimated marginal means. For the 1st retreatment a fixed retreatment yielded significantly better results than the "on-flare": mean DeltaDAS28 = -2.4 (95% CI: -3.0; -1.7) vs. -1.8 (95% CI: -3.6; -0.03), $p < 0.0001$. Similar results were found for the 2nd retreatment: mean DeltaDAS28 = -2.6 (95% CI: -3.1; -2.2) vs. -1.6 (95% CI: -1.8; -1.4), $p < 0.0001$.

Conclusion: To conclude, repeated retreatment with RTX can lead to further clinical improvement after the first course of RTX. A fixed retreatment strategy with RTX in RA seems to be more effective than the retreat 'on-flare' strategy.

Rituximab (RTX) is an anti-CD20 chimeric monoclonal antibody approved for the treatment of active Rheumatoid Arthritis (RA). Its efficacy and acceptable safety profile has been shown in large randomized controlled trials¹⁻⁴. A course of rituximab consists of two infusions of 1000mg each with two weeks interval. It is common clinical practice to retreat patients who respond to the first cycle of rituximab, but there is still no clear consensus about how often should one retreat and when.

Initially it was shown that clinical response did not correlate with B-cell depletion, as B cells were depleted in all patients after rituximab treatment, but only a proportion of patients responded to therapy⁵. This would suggest that patients who did not respond to therapy with rituximab might have a B-cell independent disease. More recent studies however have shown a correlation between depth of B-cell depletion both in the circulation and in the synovium and clinical response^{6,7}. Persistence of B-cells is associated with poorer prognosis⁸. This would suggest that retreatment with rituximab even in those patients who do not exhibit a response after the first cycle could yield good results. Indeed, while a study by Thurlings et al⁶ showed that for patients who did not exhibit clinical improvement after the first course of rituximab retreatment with rituximab was not effective, later studies showed enhanced clinical response after retreatment even in non-responders⁹.

Regarding time to retreatment, there are two main strategies: to retreat on flare or to retreat on a fixed interval, before the patient flares. Some data have suggested that the later strategy would be of preference, but that needs to be further elucidated¹⁰.

The aims of this study were: 1) to evaluate and compare the effectiveness of repeated treatment cycles with rituximab and 2) to compare retreatment 'on-flare' and fixed retreatment rituximab in a large real-life cohort of RA patients.

Methods

Patients' selection

The European Collaborative Registries for the Evaluation of Rituximab in Rheumatoid Arthritis (CERERRA) is an investigator-led, industry-supported initiative with the aim of evaluating the clinical aspects of rituximab use in patients with RA¹¹. All 12 participating European registries (Czech Republic, Denmark, Finland, the Netherlands, Norway, Portugal, Romania, Russia, Slovenia, Spain, Sweden and Switzerland) submitted fully anonymized datasets with baseline characteristics of all patients with a diagnosis of RA who had started treatment with rituximab. It is a retrospective observational cohort, but the data are collected prospectively. Data were pooled and analyzed.

The following information was collected: demographic data (age, sex); RA disease duration (from the time of diagnosis); rheumatoid factor (RF); anti-cyclic citrullinated peptide antibodies (anti-CCP); number of prior synthetic disease modifying anti-rheumatic drugs (DMARDs) and number of prior biologic DMARDs; disease activity score based on 28-joint status (DAS28) and its components (swollen joint count, tender joint count, visual analogue

scale general health and erythrocyte sedimentation rate (ESR)); functional ability based on health assessment questionnaire (HAQ); concomitant glucocorticoid and synthetic DMARD use. Information about the retreatment was also collected, such as the number of retreatment, date and reason for retreatment. The reason for retreatment was based on the rheumatologist's opinion and was either flare (deterioration of the disease) or fixed retreatment. The latter is a common clinical practice in some countries.

From the whole cohort of patients we excluded those who had no follow-up visit. These were patients who were mainly lost to follow up or patients who started treatment with RTX close to the time of the data collection and therefore had not enough time to contribute with a follow up visit. For the first analysis we identified and selected patients who received at least 4 cycles with RTX. The purpose of this was to reduce the risk for selection bias, as it is expected that mainly responders will continue with retreatment. By selecting only patients who receive at least 3 retreatments we can examine the real effectiveness of retreatment and examine the feasibility of further improvement in disease activity. Five subgroups were formed, 1st, 2nd, 3rd, 4th treatment and a fifth group (called 5th treatment) where all treatments after the 5th were pooled together.

In the second analysis, patients who received at least 1 retreatment (2 courses) with RTX and for whom information about the strategy for retreatment was available (according to the physician's opinion) were identified. The two retreatment strategies were compared by applying an adjusted mixed model analysis with DAS28 improvement as the dependent variable.

Statistical analysis

The different subgroups of patients were characterized by means of descriptive statistics. The normality of variables was tested by skewness. Normally distributed continuous variables were presented as mean \pm standard deviation (SD). Student's t-test was used to compare continuous variables while χ^2 test was used for nominal variables. The level of statistical significance was set to 5%.

Effectiveness of treatment was assessed by disease activity score (DAS28) and health assessment questionnaire (HAQ) reduction during the first 12 months from the start of each treatment. Disease activity state (based on DAS28) and EULAR response at the start (baseline) of each treatment cycle were also used to assess the feasibility of further improvement with each cycle.

The number of measurements (follow-up visits) were not the same for all patients and were not done at fixed times, as it was expected in a real-life setting. Some follow-up visits were even missing for some patients. For these reasons mixed models analysis was used, as it can naturally handle uneven spacing of repeated measurements and even missing data (as long as missingness is at random). A linear mixed-effects models with Δ DAS28 as dependent variable and treatment cycle as fixed effect was initially performed to assess the effectiveness of each treatment cycle. Time was also fitted in the model. Country and individual patient were included in the model as random variables. Different association models for the

covariance structure between the repeated measures of the primary outcomes were performed and compared using the Akaike information criterion. In a second analysis, the two retreatment strategies were compared by fitting an adjusted mixed effects model analysis to the longitudinal DAS28 for patients with complete covariate information, including country, sex, age, anti-CCP status, number of prior biologics and concomitant DMARD treatment. Time was fitted in the model.

Results

A total of 4353 RA patients started treatment with rituximab in the cohort. Of these 3718 patients had at least 1 follow-up visit and were included in the analysis. The number of patients who received at least 4 cycles of rituximab (retreated at least 3 times during the observational period) was 777. Of those 81.6% were female, 83.8% were RF positive and 78.1% were anti-CCP positive. The mean \pm SD age was 55.7 ± 12.1 years and median disease duration 11 years (IQR=6-18). Patients had failed a mean of 3.0 (SD=1.6) prior synthetic DMARDs and 1.3 (SD=1.1) prior biological DMARDs. Mean baseline (=time of 1st rituximab cycle) DAS28-ESR and HAQ was 5.7 (SD=1.4) and 1.6 (SD=0.7), respectively. The majority (80.3%) of all patients received concomitant synthetic DMARD treatment, while 66% received concomitant glucocorticoids.

Effectiveness of repeated retreatments

The total number of observations during the first 12 months from the beginning of each cycle (1st, 2nd, 3rd, 4th and 5th or more) was 2029, 2025, 1892, 1743 and 2317, respectively. The mean \pm SD DAS28-ESR improved significantly through treatment cycles: 5.0 ± 1.5 at 1st cycle; 4.3 ± 1.3 at 2nd cycle; 4.0 ± 1.4 at 3rd cycle; 3.9 ± 1.3 at 4th cycle; and 3.7 ± 1.3 at 5th or more cycle. The mean \pm SD HAQ on the contrary reduced significantly from 1st to 2nd cycle but remained stable after the second treatment cycle (mean \pm SD = 1.4 ± 0.7 , 1.3 ± 0.7 , 1.2 ± 0.7 , 1.2 ± 0.7 and 1.3 ± 0.7 from the 1st to the 5th cycle, respectively). In the mixed model analysis with DeltaDAS28 as the dependent variable, time and treatment cycle as fixed factors and country and individual patient as random factors, each treatment course was associated with significant changes in DeltaDAS28 ($p < 0.0001$). Comparison between curves revealed significant difference between all cycles. In figure 1 fixed predicted DeltaDAS28 during the first 12 months from the beginning of each treatment cycle (1st, 2nd, 3rd, 4th and 5th or more RTX cycle) is shown. In figure 2A and 2B the percentage of patients in different disease activity state and EULAR responses, respectively, at baseline (start) of each treatment cycle are shown.

'On-flare' versus 'fixed' retreatment

A total of 800 patients were retreated at least 2 times and the reason for retreatment was stated: for 616 of them the reason was flare (442 at 1st and 174 at 2nd retreatment) and for 184

of them it was a fixed retreatment (128 at 1st and 56 at 2nd retreatment). In table 1A and 1B baseline characteristics of patients in the two retreatment groups at 1st and 2nd retreatment, respectively, are summarized and compared.

Patients receiving fixed retreatment had a significantly higher (in absolute number) DeltaDAS28 ($p<0.0001$) at the start of each cycle, compared to those retreated on-flare. In the adjusted mixed model analysis, we compared the two retreatment groups for the 1st and the 2nd retreatment separately using estimated marginal means. For the 1st retreatment a fixed retreatment yielded significantly better results than the “on-flare”: mean DeltaDAS28=-2.4 (95% CI: -3.0; -1.7) vs. -1.8 (95% CI: -3.6; -0.03), $p<0.0001$. Similar results were found for the 2nd retreatment: mean DeltaDAS28=-2.6 (95% CI: -3.1; -2.2) vs. -1.6 (95% CI: -1.8; -1.4), $p<0.0001$. The evolution of the predicted DeltaDAS28 – ESR according to retreatment strategy which resulted from the adjusted mixed model analyses for the 1st and 2nd retreatment is shown in figure 3A and 3B, respectively. The retreatment strategy had a significant effect on the model.

Discussion

The results of this large, observational, international, cohort study supports the findings from earlier studies about the effectiveness of repeat treatment cycles with rituximab in RA. Patients who continue with rituximab are likely to improve further in their disease activity with repeated rituximab treatment cycles. This is a clinically relevant and important observation. Previous clinical trials and observational studies concluded similar findings^{10,12}. In our study the possibility of further improvement was demonstrated both by DAS28 reduction (figure 1) and proportion of patients in low disease activity/remission and EULAR good responders (figure 2A and 2B). By selecting only patients with at least 4 treatment cycles we minimized the risk for selection bias.

The optimal retreatment strategy remains until today unclear. As it was observed in this cohort the majority of patients were retreated on flare but there was a respectable number of patients who were retreated at a fixed interval. The results of the mixed model regression analysis suggested that a fixed retreatment approach, before a flare occurs, might lead to more favorable results. This finding is in agreement with previous findings from the MIRA registry, where patients who were retreated before they flared achieved significantly greater reduction in their disease activity compared to those retreated after they flared¹⁰. In a retrospective analysis of RA patients receiving multiple courses of rituximab a retreatment regimen based on 24-week evaluations and a treat-to-target approach was associated with better efficacy and tighter control of disease activity compared with treatment as-needed¹³.

This study has certain limitations. It is a retrospective observational cohort, although the data were collected prospectively. Patients were not randomized to the two retreatment strategy groups, and therefore the two groups were not completely balanced with regard to baseline characteristics. We tried to partially overcome this problem by adjusting for baseline differences. Significant heterogeneity between countries was observed, and country was therefore included as a random variable in the mixed model analysis.

Since patients who are retreated on fixed interval are expected to receive more treatment cycles, it would be interesting and important to know if these patients are in a higher risk for

adverse events, such as infections. The systematic collection of safety data in this study was not feasible, and therefore data on adverse events were not available. Data from other sources however have not shown any significant impact of repeated treatment cycles on safety^{13,14}.

Significant strengths on the other hand include the large number of patients, the possibility to examine different treatment strategies in different countries, the possibility of examine multiple courses of rituximab in a real-life cohort and the long follow up.

To conclude, repeated retreatment with RTX can lead to further clinical improvement after the first course of RTX. A fixed retreatment strategy with RTX in RA seems to be more effective than the retreat 'on-flare' strategy.

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Tables and figures

1st Retreatment			
	On-flare N=442	Fixed N=128	Difference
Months from BL	10.0±5.7 [442]	8.5±7.1 [128]	0.02
Age (years)	49.5±11.8 [439]	51.1±12.6 [125]	0.2
Sex (% female)	88% [442]	85% [128]	0.5
Disease Duration (years)	11.0±7.9 [439]	11.0±8.0 [125]	0.9
RF (% positive)	79% [382]	79% [104]	1.0
Anti-CCP (% positive)	78% [138]	66% [58]	0.07
N. Previous DMARDs	2.4±1.2 [430]	2.5±1.5 [119]	0.4
N. Previous biologics	0.5±0.7 [432]	0.6±0.7 [118]	0.2
Baseline DAS28	5.1±1.3 [424]	4.1±1.4 [120]	<0.0001
DAS28 at RTX start	6.3±1.0 [414]	6.1±1.2 [122]	0.03
Baseline HAQ	1.5±0.7 [355]	1.3±0.8 [90]	0.001
Concomitant DMARDs	82% [442]	92% [128]	0.02
Concomitant corticosteroids	58% [442]	46% [128]	0.004

Table 1A. Baseline (=start of 2nd RTX cycle) characteristics of patients who were retreated on flare or at fixed intervals. Number of patients with available information is shown in square brackets.

2nd Retreatment			
	On-flare N=174	Fixed N=56	Difference
Months from BL	19.9±7.0 [174]	14.4±6.6 [56]	<0.0001
Age (years)	50.3±12.3 [174]	51.3±10.8 [56]	0.6
Sex (% female)	86% [174]	91% [56]	0.5
Disease Duration (years)	11.7±8.6 [173]	11.2±8.6 [55]	0.7
RF (% positive)	82% [147]	88% [50]	0.5
Anti-CCP (% positive)	82% [56]	63% [30]	0.07
N. Previous DMARDs	2.4±1.3 [169]	2.8±1.5 [55]	0.07
N. Previous biologics	0.7±0.7 [167]	0.7±0.9 [55]	0.7
Baseline DAS28	5.2±1.3 [168]	4.0±1.3 [53]	<0.0001
DeltaDAS28 from baseline	-0.7±2.0 [163]	-2.1±1.4 [53]	<0.0001
DAS28 at RTX start	6.2±1.1 [163]	6.3±1.1 [53]	0.7
Baseline HAQ	1.6±0.7 [141]	1.4±0.7 [26]	0.1
Concomitant DMARDs	54% [174]	54% [54]	0.9
Concomitant corticosteroids	81% [174]	86% [56]	0.4

Table 1B. Baseline (=start of 3rd RTX cycle) characteristics of patients who were retreated on flare or at fixed intervals. Number of patients with available information is shown in square brackets.

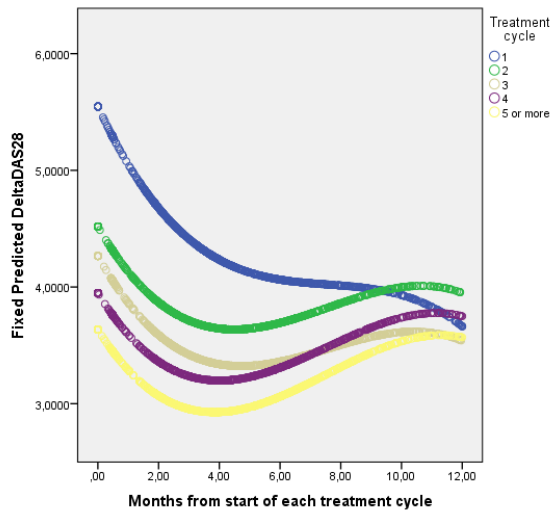


Figure 1. Reduction of DAS28-ESR (DeltaDAS28) during the first 12 months after start of 1st, 2nd, 3rd, 4th and 5th or more treatment cycle for patients who received at least 4 RTX treatment cycles (5th treatment = all treatments after the 4th pooled together). Significant DeltaDAS28 was observed for all treatment cycles and the difference between curves was significant in all comparisons ($p < 0.0001$).

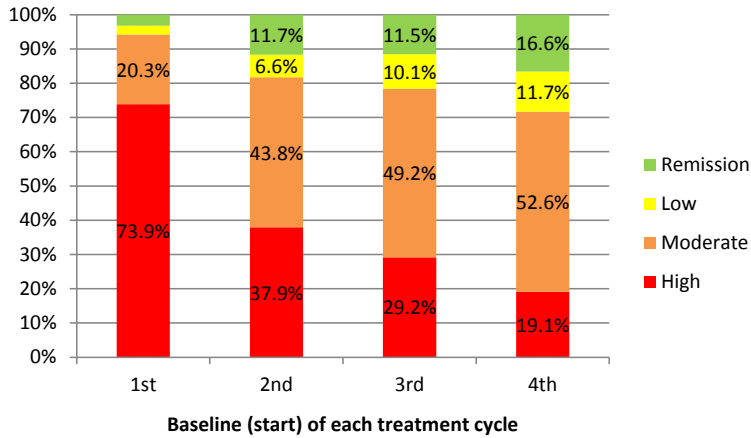


Figure 2A. Disease activity state based on DAS28 at baseline (visit 1) of each treatment cycle for patients who received at least 4 RTX treatment cycles. A significant reduction of the proportion of patients in high disease activity is observed parallel to an increase of the proportion of patients in low disease activity and remission.

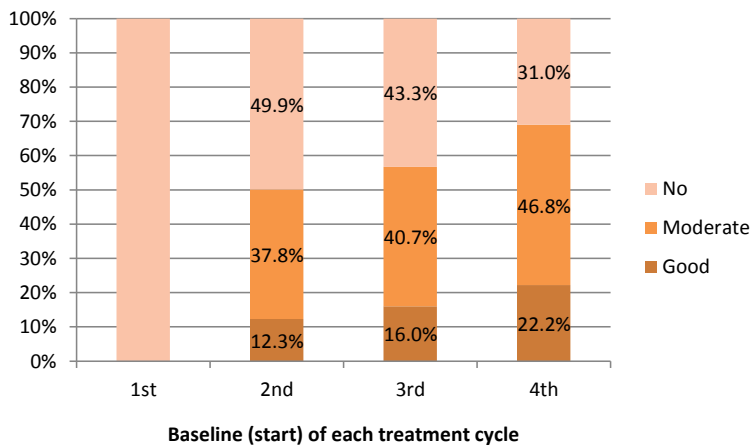


Figure 2B. EULAR response rates at baseline (visit 1) of each treatment cycle for patients who received at least 4 RTX treatment cycles. A significant reduction of the proportion of non-responders was observed parallel to an increase of the proportion of EULAR good and moderate responders.

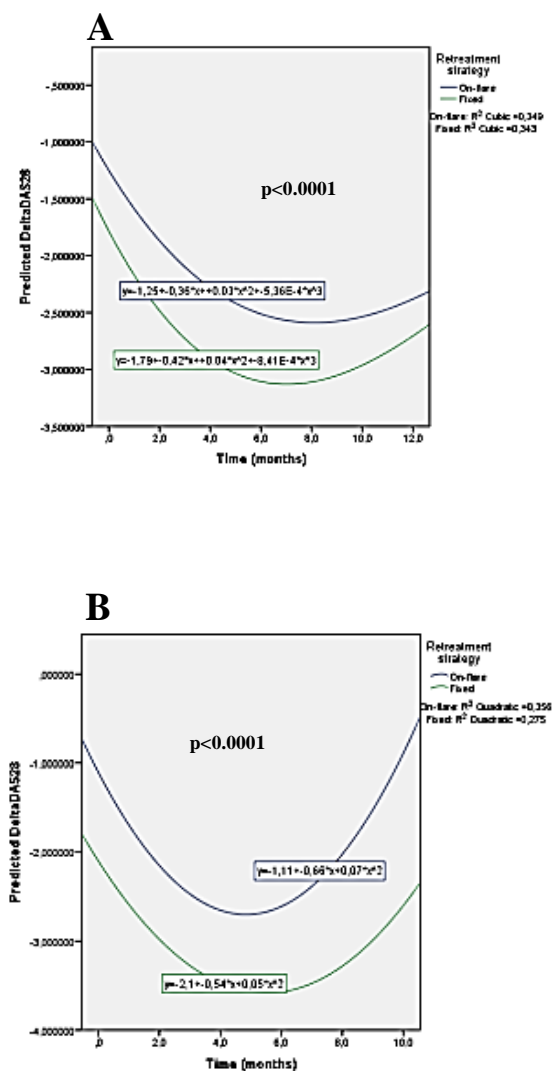


Figure 3A and 3B. Predicted DeltaDAS28 – ESR according to retreatment strategy resulted from the adjusted mixed model analyses for the 1st (figure 3A) and 2nd (figure 3B) retreatment. The models were adjusted for age, sex and baseline characteristics that differed significantly between groups. The retreatment strategy had a significant effect on the model.

V

EXTENDED REPORT

Effectiveness of TNF inhibitor switch in RA: results from the national Swedish register

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ABSTRACT

Background Switching to a second tumour necrosis factor inhibitor (TNFi) after discontinuation of a first in rheumatoid arthritis (RA) is a common strategy. The reason for the switch from the first TNFi could potentially influence the response to therapy. Data on direct comparisons between TNFi after switching are limited.

Methods The national Swedish register was used. RA patients who switched to a second TNFi (infliximab, etanercept or adalimumab) after failure of a TNFi as first-ever biologic were identified. Effectiveness of treatment was compared across the three drugs according to the first TNFi used, the reason for discontinuing and the drug survival. Drug survival across TNFi used as second biologic was compared.

Results Half of all patients starting infliximab, adalimumab or etanercept during the period 2005–2012 discontinued treatment for various reasons. Of these patients, a third switched within 2 months to a second TNFi (infliximab, etanercept or adalimumab). Around 35% of all patients achieved low disease activity or remission at 6 months. Regarding the switching strategy, best results were observed among patients who switched from infliximab to etanercept because of (secondary) inefficacy. Etanercept as second TNFi was associated with longer drug survival compared with infliximab.

Conclusions Switching to a second TNFi after the failure of the first may lead to good clinical results. The inter-drug differences in drug survival on the second TNFi mirror those reported previously for the first TNFi, suggesting that these differences are not solely due to channelling bias.

INTRODUCTION

The first biologic agents to be approved for the treatment of active rheumatoid arthritis (RA), refractory to conventional antirheumatic therapy, were the tumour necrosis factor inhibitors (TNFis) etanercept (ETA), infliximab (INF) and adalimumab (ADA).^{1–3} ETA is a soluble TNF receptor, INF a chimeric anti-TNF monoclonal antibody and ADA a fully human anti-TNF monoclonal antibody. In the last 3 years, two new anti-TNF monoclonal antibodies have become available, namely, certolizumab pegol and golimumab.^{4–6}

The significant efficacy and acceptable safety profile of these drugs have been demonstrated in large randomised controlled clinical trials.^{1–11} However, it has also been shown that a significant number of patients discontinue treatment for various reasons, mainly due to inefficacy or intolerance. Indeed, a number of

studies from clinical practice indicate that as many as 50% of all patients discontinue their TNFi treatment during the first 3 years.^{12–14}

In clinical practice, switching to a second TNFi is common, the rationale being that the different TNFis differ in their molecular structure, immunological action, immunogenicity and pharmacokinetics. Both clinical trials and epidemiological studies have demonstrated the efficacy of TNF inhibition after TNF failure.^{15–20} To optimise switching between TNFis in clinical practice, more information is needed on whether factors such as reason for switching (inefficacy or intolerance of the first TNFi) or type of the first TNFi influence the response to the second TNFi. Previous studies have examined the effectiveness of switching from the first TNFi.^{13 14 20 21} However, no study to our knowledge has hitherto compared specific switching strategies (switching between individual TNFis).

In many observational studies, slightly better retention rates and effectiveness have been reported for ETA than for ADA and INF, but there is some uncertainty whether this superiority reflects channelling bias or a true difference.^{14 22} One way to ‘disconnect’ such channelling from drug-related effects is to make inter-drug comparisons of drug survival and effectiveness of individual TNFis used as second TNFi (ie, when the initial channelling was to another TNFi).

The aims of this study were therefore to (1) assess switching from first to second TNFi under different circumstances (according to the reason for discontinuation and the type of the first TNFi) trying to identify an optimal switching strategy and (2) examine drug survival of ADA, ETA and INF when used as second TNFi after switching from a first TNFi in patients with RA identified in clinical practice.

METHODS

Study population

Data from the nationwide Swedish Biologics Register (Anti-Rheumatic Therapy in Sweden (ARTIS)) were used. To this register, data on adult patients prescribed biologic agents for the treatment of rheumatic diseases in Sweden have been collected since 1999. The coverage of the ARTIS database has been estimated to be nearly 90% of all eligible patients with RA.^{23 24} Most RA patients in Sweden start an anti-TNF after failure of at least one synthetic disease modifying antirheumatic drug.

Patients included in this observational cohort study had a diagnosis of RA and had started

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treatment with a 'first-ever' used TNFi (INF, ETA or ADA) during the period 01 January 2005–01 September 2012. We chose to include patients who started TNFi treatment after 2005 since all three TNFis were available and switching from one TNFi to another was common in clinical practice. Among these, we further identified those patients who switched to INF, ETA or ADA as second TNFi. Patients switching to another biologic (rituximab, tocilizumab or abatacept), to one of the newest TNFis (golimumab, certolizumab pegol), or those who stopped and restarted the same TNFi were excluded from the analysis. Although many patients who discontinue a first biologic may eventually start a second, for the purpose of this study, we defined 'switching' as starting a second and different TNFi within 2 months from the date of discontinuation of the first TNFi. By setting this time frame, we eliminated the risk that a patient would start another non-biologic disease modifying anti-rheumatic drug before switching to a second TNFi, as the assessment of treatment efficacy takes place after 3 months. Patients who did not have enough follow-up time (started the second TNFi within 8 months from the last observation date (01 September 2012)) were also excluded from this analysis. Four different reasons for discontinuation of the first TNFi were considered: primary inefficacy (lack of efficacy, including partial efficacy), secondary inefficacy (loss of efficacy), intolerance and other (pregnancy, patient's or physician's decision, inactive disease, death, unknown).

The following information was collected: demographic data (age, sex); RA disease duration (from the time of diagnosis); rheumatoid factor; type of first TNFi, date and reason for discontinuation of the first TNFi; date of initiating therapy with the second TNFi and, if discontinued, date and reason; disease activity score based on 28-joint status (DAS28) and its components (swollen joint count, tender joint count, visual analogue scale general health and erythrocyte sedimentation rate); and functional ability based on health assessment questionnaire. The reason for discontinuation was based on the rheumatologist's opinion and was recorded according to a predefined list of different options.

Effectiveness of treatment was assessed by DAS28 change (Δ DAS28) between baseline and 6 months (150–240 days from baseline), the percentage of patients achieving low disease activity or remission (according to DAS28 status: low disease activity= $\text{DAS28} \leq 3.2$, remission= $\text{DAS28} \leq 2.6$) as well as the percentage of patients achieving DAS28 improvement ≥ 1.2 at 6 months. Baseline was defined as start of second TNFi. Drug survival of the three TNFis as second biologic was also assessed.

Statistical analysis

Three exposure categories of interest were defined: patients who switched to INF, ETA or ADA after the failure of either of these drugs used as the first-ever TNFi. Baseline characteristics across the three groups were summarised and compared. The normality of all continuous variables was tested by skewness. Variables which were similar to normal distribution were presented as mean \pm SD, while non-normally distributed variables were presented as median with IQR. One-way analysis of variance was used to compare continuous variables (with a distribution similar to normal) followed by Bonferroni test for post hoc comparisons between the groups, while χ^2 test was used for nominal variables. Kruskal–Wallis test was used to compare non-normally distributed variables. The level of statistical significance was set to 5%.

We used two time frames for follow-up, 3 and 6 months. To comply with clinical practice, the time window was 30–150 days

from baseline for the 3-month visit and 150–240 days from baseline for the 6-month visit. The median time to first visit in ARTIS is 4.5 months after the start date. We prioritised 6 months data over 3 months data, when available, but when no such data were available, we used data from the 3-month visit. Non-responder imputation (defined as high or moderate disease activity at 6 months) was used for patients switching to another biologic during the follow-up period and for patients who did not have a follow-up visit but for whom the stated reason for stopping treatment was primary or secondary inefficacy. Three main analyses were performed. (1) The effectiveness of INF, ETA and ADA as second TNFi was directly compared in a first analysis. Stratification for baseline DAS28 (high baseline disease activity defined as $\text{DAS28} \geq 5.1$ vs non-high with $\text{DAS28} < 5.1$) was performed to investigate effect modification by disease activity, that is, any inter-drug difference would differ for different levels of baseline disease activity. (2) The effectiveness of the three TNFis was assessed as a function of the first TNFi. In total, six different switching strategies were thus compared (first ADA then ETA, first ADA then INF, first ETA then ADA, etc). (3) Effectiveness of the second TNFi was assessed as a function of the reason for discontinuation of the first TNFi. Four different reasons for discontinuation of the first TNFi were considered: intolerance, primary inefficacy (lack of efficacy, including partial efficacy), secondary inefficacy (loss of efficacy) and other (pregnancy, patient's or physician's decision, inactive disease, death, unknown).

Kaplan–Meyer curves were plotted to determine continuation rates for the second TNFi during the first 2 years following switch. Curves were compared with the Log-Rank test. Discontinuation of treatment (for the reasons described above) was considered an event. Continuation of treatment at the time of data collection was treated as censored observations during the analysis.

RESULTS

We identified 7052 patients with a diagnosis of RA who started treatment with a first-ever TNFi (INF, ETA or ADA) during the period 01 January 2005–01 September 2012: 2174 with INF, 3076 with ETA and 1802 with ADA. During the same time-period, 50% of these patients discontinued their TNFi treatment. Of these, 2649 (38%) patients started a second biologic, 1457 to a second TNFi. In the final study population, 952 patients who switched (according to our definition) to a second TNFi and had long enough follow-up time were included (74 switched to INF, 448 to ETA and 430 to ADA within 2 months after discontinuing the first TNFi). In figure 1, the flow chart of patients is shown.

Baseline for our analyses was defined as the time of start of the second TNFi. Patients in the three groups were quite well balanced regarding baseline characteristics, except for baseline DAS28 which was significantly lower in the ADA group compared with the other two groups (table 1). Regarding baseline DAS28 status, the completeness of available data was 80% for INF, 83% for ETA and 78% for ADA. Regarding available DAS28 status/ Δ DAS28 at 6 months, completeness of available data was 81/64% for INF, 86/74% for ETA and 86/73% for ADA. The percentage of missing data at 3 and 6 months was similar across the three TNFi groups.

Effectiveness of second TNFi, per drug

At 6 months patients in all three groups achieved significant reductions in DAS28: Δ DAS28 0–6 months= -1.1 ± 1.5 [n=38] for INF, -1.4 ± 1.6 [n=275] for ETA and -0.8 ± 1.5 [n=244]

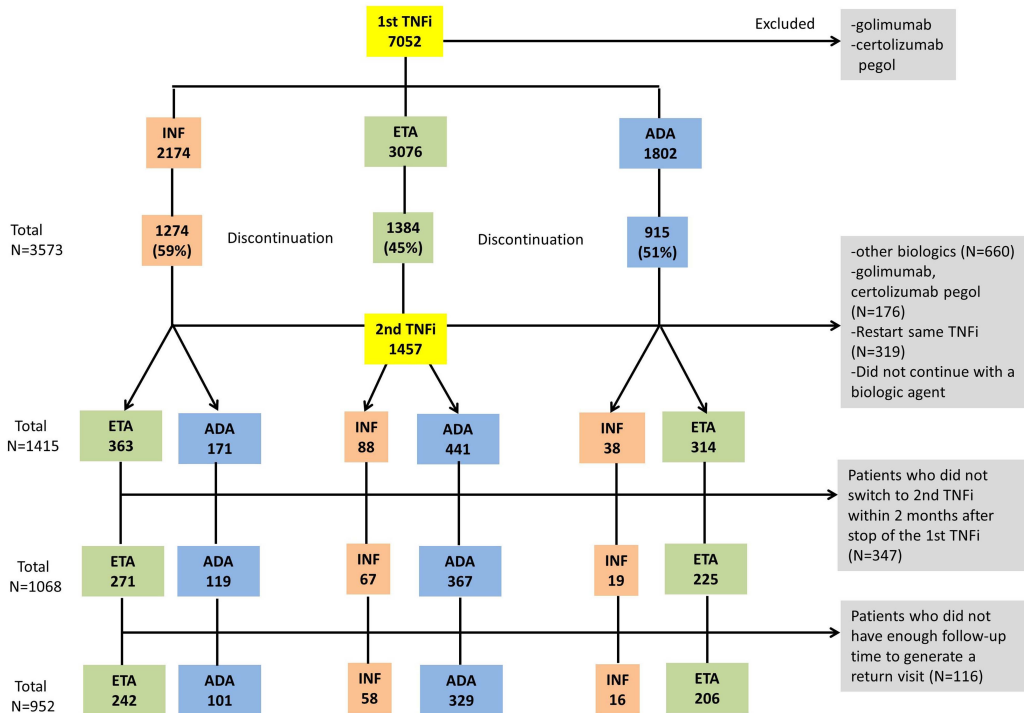


Figure 1 Flow chart of patients (selection as described in the methods). 7052 Swedish RA patients started treatment with infliximab (INF), etanercept (ETA) or adalimumab (ADA) as 1st TNFi during 2005-2012. Around 50% discontinued treatment during the observational period (number and percentage of patients discontinued indicated in the boxes). 1457 patients continued with a 2nd TNFi, of which 1068 'switched' to INF, ETA or ADA within 2 months after discontinuing the 1st TNFi. (Grey boxes: patients excluded).

for ADA. The inter-drug difference was statistically significant between ETA and ADA ($p<0.0001$). After adjustment for baseline DAS28, this difference remained significant ($p=0.04$). The percentage of patients who achieved $\Delta\text{DAS28}\geq 1.2$ was 47% (18/38) for INF, 55% (151/275) for ETA and 36% (87/244) for ADA ($p<0.0001$ ETA vs ADA). The percentage of patients with low disease activity or remission at 6 months, however, was similar for all groups: 37% (18/49) for INF, 34% (113/332) for ETA and 35% (103/294) for ADA. When stratified by baseline DAS28, the ETA group achieved numerically higher but statistically not significantly different DAS28 reductions than ADA (table 2).

Effectiveness of second TNFi as a function of the type of first TNFi

In the second analysis, we examined the effectiveness of the second TNFi taking into consideration the TNFi switched from. The six possible switching groups were distributed as follows: from ADA to ETA (N=206), from ADA to INF (N=16), from ETA to ADA (N=329), from ETA to INF (N=58), from INF to ADA (N=101) and from INF to ETA (N=242). The effectiveness observed for each switching strategy is shown in table 3. For the two largest groups, statistically greater ΔDAS28 was observed in the group switching from INF to ETA than for the group switching from ETA to ADA ($p<0.0001$, after adjustment for baseline DAS28, $p=0.004$). Overall better results were observed for the INF→ETA and the ADA→ETA groups

compared with ETA→ADA (the two reciprocal groups, ETA→INF and ADA→INF, were too small to allow any comparisons).

Effectiveness of second TNFi by reason for discontinuation of first TNFi

In the third analysis, we examined the effectiveness of the second TNFi according to the reason for switch from the first TNFi. The majority of patients switched to the second TNFi after lack or loss of efficacy of the first TNFi (66%), while 17.4% of patients switched due to intolerance. The number of patients as well as effectiveness data according to the reason for switch is shown in table 4. Overall numerically higher ΔDAS28 at 6 months was observed after loss of efficacy (mean ΔDAS28 at 6 months = -1.4 ± 1.6) than after lack of efficacy (mean ΔDAS28 = -1.2 ± 1.6) or intolerance (mean ΔDAS28 = -1.1 ± 1.5) to the first TNFi. Significantly higher rates of low disease activity/remission at 6 months were achieved when the reason for switch was secondary inefficacy (40%) or intolerance (39%) than primary inefficacy (26%) ($p<0.0001$). The best responses were observed when switching to ETA after losing efficacy of ADA or INF as the first TNFi.

Drug survival on the second TNFi, per drug

During the first 24 months after switching to the second TNFi, 567 patients (60%) discontinued their second TNFi: 46 out of 74 in the INF group (62%), 257 out of 448 (57%) in the ETA group

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Table 1 Characteristics for Swedish patients with RA starting a second TNFi (INF, ETA or ADA) during 2006–2012 at the time-point of their second TNFi start

	INF N=74	ETA N=448	ADA N=430	Difference among groups (p value)	Post hoc comparisons between groups
Age in years (mean±SD)	52.7±15.0 (74)	53.8±13.0 (448)	54.4±13.4 (430)	0.56	
Sex (% female)	77.0% (74)	77.0% [448]	77.2% (430)	0.99	
Disease duration (median, IQR)	9.9 (3.2–15.1) (67)	8.4 (3.7–15.9) (408)	8.6 (4.3–16.8) (381)	0.66	
RF (% positive)	85.9% (71)	81.1% (429)	74.6% (410)	0.02	INF vs ADA ETA vs ADA
Baseline DAS28 (mean±SD)	5.0±1.3 (59)	5.1±1.4 (372)	4.7±1.4 (335)	0.001	ETA vs ADA
Baseline HAQ (mean±SD)	1.2±0.7 (63)	1.2±0.6 (385)	1.1±0.6 (363)	0.38	
Concomitant DMARDs (% yes)	86.5% (74)	76.8% (448)	69.5% (430)	0.002	INF vs ADA ETA vs ADA
Concomitant MTX (%)	82.4%	65.0%	59.5%	0.001	INF vs ETA INF vs ADA ETA vs ADA
Concomitant Glucocorticoids (% yes)	41.9% (74)	52.9% (448)	50.5% (430)	0.21	

Number in brackets indicates the number of patients with available information for each variable. One-way analysis of variance was used to compare continuous variables followed by Bonferroni test for post hoc comparisons between the groups, while χ^2 test was used for nominal variables. Kruskal–Wallis test was used to compare non-normally distributed continuous variables across groups.

ADA, adalimumab; DAS28, disease activity score based on 28-joint status; DMARD, disease modifying antirheumatic drug; ETA, etanercept; HAQ, health assessment questionnaire; INF, infliximab; MTX, methotrexate; RA, rheumatoid arthritis; RF, rheumatoid factor; TNFi, tumour necrosis factor inhibitor.

and 264 out of 430 (61%) in the ADA group (figure 2). The median (95% CI) survival time for INF, ETA and ADA was 14 (7–21), 24 (16–32) and 16 (9–23) months, respectively. Since the survival curves crossed at approximately 8 months (figure 2), two

separate analyses were performed; up to 8 months following switch, no significant differences in drug survival among ADA, ETA and INF were observed, but for the second time-period significant differences were observed (figure 2).

Table 2 Effectiveness of second TNF inhibitor (INF, ETA and ADA) based on Δ DAS28, % of patients with low disease activity or remission at 6 months and % of patients achieving Δ DAS28 of at least 1.2 at 6 months, stratified by disease activity at baseline (high vs no high)

No high disease activity at baseline	INF N=31	ETA N=184	ADA N=199	Difference between groups (p value)	Post hoc comparisons between groups
DAS28 baseline	4.0±0.8 (31)	4.0±0.9 (184)	3.8±1.0 (199)	0.12	
DAS28 6 months	3.4±1.6 (22)	3.3±1.3 (125)	3.4±1.2 (145)	0.81	
Δ DAS28 6 months	−0.6±1.4 (22)	−0.8±1.3 (125)	−0.4±1.3 (145)	0.09	0.09 ETA vs ADA
% Low disease activity or remission 6 m	52.2% (23)	44.3% (131)	46.6% (146)	0.77	
% Δ DAS28≥1.2	31.8% (22)	37.6% (125)	26.9% (145)	0.17	
High disease activity at baseline	INF N=28	ETA N=188	ADA N=136	Difference between groups (p value)	Post hoc comparisons between groups
DAS28 baseline	6.1±0.6 (28)	6.2±0.8 (188)	6.0±0.7 (136)	0.13	
DAS28 6 months	4.3±1.1 (16)	4.2±1.5 (150)	4.6±1.5 (99)	0.19	
Δ DAS28 6 months	−1.8±1.3 (16)	−1.9±1.5 (150)	−1.4±1.6 (99)	0.05	0.05 ETA vs ADA
% Low disease activity or remission 6 m	18.8% (16)	24.8% (153)	19.8% (101)	0.60	
% Δ DAS28≥1.2	68.8% (16)	69.3% (150)	48.5% (99)	0.003	0.003 ETA vs ADA

ADA, adalimumab; DAS28, disease activity score based on 28-joint status; Δ DAS28, DAS28 change; ETA, etanercept; INF, infliximab; TNF, tumour necrosis factor. Bold typeface indicates statistical significance.

Table 3 Effectiveness of six different switching strategies (first TNFi→second TNFi) based on Δ DAS28 after 6 months, % of patients with low disease activity or remission at 6 months and % of patients achieving Δ DAS28 of at least 1.2 at 6 months

	First TNFi→second TNFi					
	INF→ETA N=242	INF→ADA N=101	ETA→INF N=58	ETA→ADA N=329	ADA→INF N=16	ADA→ETA N=206
DAS28 baseline*	5.3±1.4 (197)	4.7±1.4 (78)	5.1±1.3 (45)	4.7±1.4 (257)	4.8±1.5 (14)	4.9±1.3 (175)
DAS28 6 months**	3.8±1.5 (180)	3.3±1.5 (64)	3.7±1.5 (38)	4.0±1.5 (225)	3.6±1.5 (10)	3.9±1.5 (141)
Δ DAS28 6 months***	-1.6±1.5 (150)	-1.2±1.6 (48)	-1.2±1.6 (30)	-0.7±1.5 (196)	-0.6±0.9 (8)	-1.2±1.6 (125)
% Low disease activity or remission at 6 months	38.0% (184)	47.0% (66)	31.6% (38)	31.6% [228]	54.5% (11)	29.1% [148]
% With DAS28 improvement ≥ 1.2 at 6 months	58.7% (150)	45.8% (48)	56.7% (30)	33.2% (196)	12.5% (8)	50.4% (125)

*p<0.0001 INF→ETA vs ETA→ADA, p=0.04 INF→ETA vs INF→ADA.

**p=0.01 INF→ADA vs ETA→ADA.

***p<0.0001 INF→ETA vs ETA→ADA (remained significant after correction for baseline DAS28, p=0.004).

ADA, adalimumab; DAS28, disease activity score based on 28-joint status; Δ DAS28, DAS28 change; ETA, etanercept; INF, infliximab; TNFi, tumour necrosis factor inhibitor.

DISCUSSION

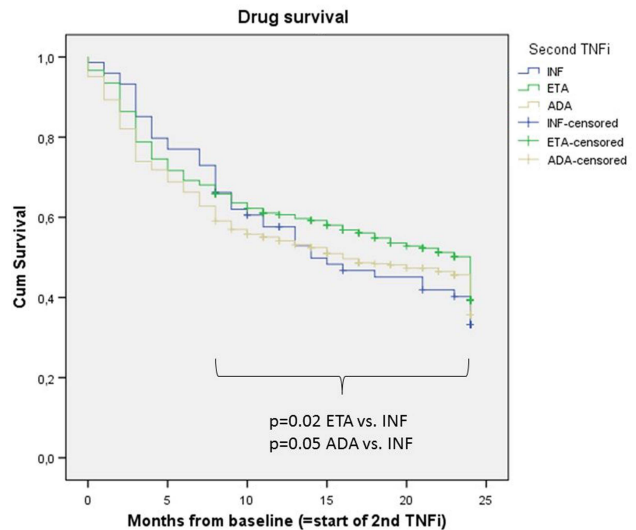
The results of our study support the findings from previous studies that switching to a second TNFi may lead to significant clinical improvements, with almost 40% of patients achieving low disease activity or remission, regardless of the specific TNFi.

Because of the real-world setting, our findings must be interpreted in light of a number of potential or real differences among the three groups of switchers. As shown in table 1, most baseline demographic and disease variables were quite similar across groups. However, baseline disease activity was significantly higher for those starting ETA as their second TNFi than ADA, which (under the assumption that the capacity for DAS28 reduction is non-linear, or simply through regression to the mean) might explain the greater improvement in DAS28 for ETA as second TNFi compared with ADA. Indeed, when stratified by baseline disease activity, we observed overall greater reductions in DAS28 among those patients with a disease activity score above 5.1 at the time point of the second TNFi start

than in the non-high disease activity group, but, as expected, a higher percentage of patients achieving low disease activity/remission in the latter. ETA achieved numerically but not significantly better improvements than ADA, which might represent a true effect but also reflect limited statistical precision. The difference was not large enough to assure a true clinical difference.

Overall better results were achieved with the second TNFi after loss of efficacy or intolerance to first TNFi than after lack of efficacy of the first TNFi, supporting the results from previous studies.^{18 20} This observation is rational and supports the hypothesis that for patients who do not respond to TNFi (primary inefficacy), TNF might not play that important role in their disease and a second TNFi would yield only modest results. Best responses were observed when switching to ETA after losing efficacy of ADA or INF as the first TNFi. The production of antidrug antibodies, drug immunogenicity, has been proposed as one possible mechanism behind inefficacy.^{25–28} Secondary inefficacy to a first TNFi might be due to development of antidrug antibodies,

Figure 2 Drug survival during the first 2 years of treatment for INF, ETA and ADA as 2nd TNFi. Significant difference was observed between INF and ETA as well as INF and ADA during the period 8–24 months (p=0.02 ETA vs. INF, p=0.05 ADA vs. INF by Log-rank test).



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Table 4 Effectiveness of the second TNFi (INF, ETA, ADA) at 6 months by reason for switching (primary and secondary inefficacy, intolerance, other)

Reason for discontinuation of first TNFi	Primary inefficacy			Secondary inefficacy			Intolerance			Other		
	INF [23]	ETA [133]	ADA [130]	INF [29]	ETA [165]	ADA [143]	INF [7]	ETA [87]	ADA [74]	INF [15]	ETA [61]	ADA [79]
DAS28 baseline	5.2±1.3 (18)	5.5±1.2* (109)	5.1±1.2 (103)	4.9±1.2 (23)	5.1±1.2 (143)	4.8±1.3 (110)	4.7±1.5 (7)	5.1±1.7 (70)	4.4±1.5 (56)	4.9±1.6 (11)	4.4±1.5 (49)	4.0±1.5 (63)
DAS28 6 months	4.0±1.4 (17)	4.1±1.5 (101)	4.3±1.4 (92)	3.2±1.5 (18)	3.6±1.5 (123)	3.9±1.5 (96)	3.3±0.8 (3)	3.9±1.6 (57)	3.4±1.6 (50)	4.2±1.5 (10)	3.6±1.1 (38)	3.5±1.2 (49)
ΔDAS28 6 months	-1.1±1.2 (13)	-1.5±1.5** (85)	-0.8±1.3 (79)	-1.3±1.1 (14)	-1.6±1.5*** (109)	-1.0±1.6 (79)	-1.3±1.2 (3)	-1.2±1.6 (50)	-1.0±1.7 (41)	-0.6±2.3 (8)	-0.9±1.6 (30)	-0.5±1.6 (43)
% Low disease activity of remission	29.4% (17)	27.2% (103)	21.5% (93)	44.4% (18)	42.3% (130)	39.8% (98)	66.7% (3)	29.8% (57)	45.1% (51)	27.3% (11)	32.5% (40)	42.0% (50)
% With DAS28 improvement ≥1.2	46.2% (13)	52.9%† (85)	34.2% (79)	42.9% (14)	61.5%† (109)	36.7% (79)	66.7% (3)	56.0% (50)	39.0% (41)	50% (8)	36.7% (30)	30.2% (43)

The number of patients in each subgroup is shown in brackets.

*p=0.08 ETA vs ADA.

**p=0.003 ETA vs ADA, p=0.02 after correction for baseline DAS28.

***p=0.03 ETA vs ADA, p=0.02 after correction for baseline DAS28.

†p=0.05 ETA vs ADA.

‡p=0.003 ETA vs ADA.

ADA, adalimumab; DAS28, disease activity score based on 28-joint status; ΔDAS28, DAS28 change; ETA, etanercept; INF, infliximab; TNFi, tumour necrosis factor inhibitor.

and non-responders in that case might benefit from a switch to a less immunogenic drug, such as ETA.

When different switching strategies were compared, overall better results were observed for patients who switched from INF or ADA (monoclonal antibodies) to ETA than the other way round. We acknowledge, however, that some of the switching strategies included a too limited number of patients to allow for meaningful interpretation. The observed difference was present mainly when the reason for switching was inefficacy rather than intolerance (see online supplementary file). A clinical interpretation of this observation might be that after the failure of a monoclonal anti-TNF antibody one could consider switching to ETA, but when a patient has failed ETA as first TNFi, the choice of a monoclonal antibody as second TNFi might not have the same potential to lead to a clinically significant improvement. Indeed, other studies have shown that in the latter case it might be more beneficial to change to a biologic of a different mechanism of action.^{29 30} This might imply an underlying mechanism that could explain this finding: as it is known, ETA competitively inhibits the binding of both TNF and lymphotoxin-α to cell surface TNF receptors, rendering TNF biologically inactive,³¹ while INF and ADA bind and neutralise both soluble and membrane-bound TNF but not lymphotoxin. Lymphotoxin plays a crucial role in chronic inflammation.³² Thus, failing a monoclonal antibody (especially because of inefficacy) a patient might respond well to ETA, and the reason could be the binding of lymphotoxin.³³ This remains, however, a hypothesis.

A limitation of this study was the observational cohort study design, as patients were not randomly placed in the treatment groups. The risk of selection bias is therefore present. The treatment groups under comparison were not entirely balanced for baseline characteristics and some significant differences were observed, which might introduce the risk for confounding. Other and unknown baseline factors may also differ between the drugs. Indeed and as mentioned in the Introduction section, confounding by indication may be responsible for some of the previously observed differences in drug survival between ETA and ADA/INF as first TNFi. In this study, we could limit some of such confounding by indicating by assessing drug survival on these drugs when used as the second TNFi (hence, when patients starting ETA were recruited from a pool of patients who had initially all be channelled to treatment with INF or ADA, and vice versa). We found some differences in favour of ETA as second TNFi, similar to what has been shown in previous analyses of first TNFi, suggesting that it is more possible that these results represent true difference and are not entirely due to confounding by indication related to the choice of the first TNFi. The clinical relevance of the observed difference is, however, unclear. Other differences between TNFis, such as route of administration, have to be taken into account. INF is given intravenously, which might influence negatively its retention rate. Such factors limit the possibility of comparing these agents using retention rates as surrogates for effectiveness. We tried to partially overcome the problem with selection bias by first adjusting for baseline DAS28 and then by stratifying according to disease activity (high vs low).

The limited number of patients in some of the comparisons was a challenge. Some numerical differences did not reach statistical significance, and this might be due to lack of power. Missingness is a common problem in register-based observational studies. In our study, missingness did not differ between drugs. Due to the limitations described above, however, the results of this study have to be interpreted with caution.

In summary, after the failure of the first TNFi, up to 40% of patients switching to a second TNFi may achieve low disease activity or remission. After the failure of a monoclonal antibody as first TNFi because of inefficacy, switching to ETA yielded good clinical results, but not the other way round. ETA was associated with longer drug survival compared with INF as second TNFi. The inter-drug differences in drug survival on the second TNFi mirror those reported previously for the first TNFi, suggesting that these differences are not solely due to channelling bias.

Collaborators The ARTIS (Anti-Rheumatic Therapy in Sweden) group: Johan Askling, Eva Baecklund, Lars Cöster, Lars Erik Kristensen, Nils Feltelius, Helena Forsblad d'Elia, Pierre Geborek, Lennart Jacobsson, Lars Klareskog, Staffan Lindblad, Martin Neovius, Solbritt Rantapää-Dahlqvist and Ronald van Vollenhoven.

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Effectiveness of TNF inhibitor switch in RA: results from the national Swedish register

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Rituximab versus anti-TNF in patients who previously failed one TNF inhibitor in an observational cohort

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Objective: The purpose of this study was to characterize and compare responses in patients who had failed one tumour necrosis factor (TNF) inhibitor when switching to another TNF inhibitor or rituximab (RTX).

Methods: The Stockholm TNF follow-up registry (STURE) was used. Treatment results at 6 months were analysed by (i) the biologic used, (ii) the type of anti-TNF switch, and (iii) the reason for discontinuation (inefficacy or intolerance).

Results: A total of 328 patients who failed an anti-TNF switched to an alternative biologic, 69 to RTX, 161 to an anti-TNF monoclonal antibody (mAb), and 98 to etanercept (ETA). Significant reductions in the 28-joint Disease Activity Score (DAS28) at 6 months were observed for all groups. The mean \pm SD reduction in DAS28 was 1.70 ± 1.18 for RTX, 1.40 ± 1.51 for ETA, and 0.67 ± 1.36 for mAb, the difference being statistically significant between RTX and mAb ($p < 0.0001$). For patients who had failed ETA, RTX led to significantly greater DAS28 reductions than mAb ($p = 0.01$). When the reason for discontinuation of the previous anti-TNF was intolerance or secondary inefficacy, RTX led to significantly greater DAS28 reduction compared to mAb and ETA ($p = 0.01$ and $p = 0.03$, respectively).

Conclusions: In this observational cohort, patients who failed one anti-TNF had better overall results when treated with RTX than with a subsequent anti-TNF mAb. Having failed ETA, RTX yielded greater DAS28 reductions and European League Against Rheumatism (EULAR) responses than mAb. The advantage of RTX was most clearly seen in patients who had failed anti-TNF because of intolerance or secondary inefficacy.

An increasing number of biological therapeutic agents for rheumatoid arthritis (RA) are now available. Besides the tumour necrosis factor (TNF) blocking agents [infliximab, etanercept (ETA), adalimumab, and the newest golimumab and certolizumab pegol], the efficacy of other biological therapies has also been established, including the B-cell depleting agent rituximab (RTX), the interleukin-6 inhibitor tocilizumab, and the inhibitor of T-cell co-stimulation abatacept (1–10). Not every patient will benefit from all of these therapies (11), and the question that has been raised during the past few years is which subgroup of RA patients would be the ‘target group’ for each biological agent.

Various research articles have shown that, after the failure of one TNF antagonist, switching to an alternative anti-TNF can provide clinical benefit for some patients (12–20). However, switching to an agent with a different mechanism of action after the failure of a TNF antagonist might seem more logical. Finckh et al examined the efficacy of RTX in comparison to that of an alternative anti-TNF agent in patients with inadequate response to

one or more anti-TNFs, and reached the conclusion that the former biological agent led to better results (21).

The aim of this observational study was to determine whether patients who failed one TNF inhibitor achieved better results when switching to a second TNF inhibitor, a monoclonal antibody (mAb), the soluble receptor ETA, or RTX. The potential influence of the type of anti-TNF switch and the relationship between the reason for discontinuation of the previous agent and the efficacy of the next treatment were also examined.

Methods

The Stockholm TNF follow-up registry (STURE) database collects efficacy data for all patients starting biological treatments at the major hospitals in Stockholm, as part of the nationwide registry of AntiRheumatic Therapies in Sweden (ARTIS). The design of the study was approved by local ethical committees. The assessments are performed at baseline, 3, 6, and 12 months, and annually thereafter, and include the American College of Rheumatology (ACR) core outcomes [the 28 swollen (SJC) and tender joint count (TJC), visual analogue scales (VAS) for global health and for pain, the Health Assessment Questionnaire (HAQ) disability index, erythrocyte sedimentation rate (ESR), C-reactive

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protein (CRP)], the DAS28 score, and record of concurrent medications.

Patients with a diagnosis of RA who started treatment with a TNF inhibitor between 1 January 2005 and 31 December 2010 were included in the cohort. Patients who failed one TNF inhibitor (infliximab, adalimumab, ETA) for various reasons and switched to an anti-TNF monoclonal antibody (mAb: infliximab or adalimumab), the soluble receptor ETA, or RTX were identified and included in the analysis. Of duplicate segments with the same anti-TNF, only the first one was used. The efficacy of each treatment was assessed by DAS28 improvement and European League Against Rheumatism (EULAR) response (Good, Moderate, None) at 6 (\pm 1) months after treatment start. For those patients with no available DAS28 value at 6 months, the 3-month DAS28 was used [as last observation carried forward (LOCF)], if they were still on treatment. Treatment results at 6 months were analysed by (i) the biologic used; (ii) the type of anti-TNF switch, and (iii) the reason for discontinuation of the previous biologic (primary inefficacy, including complete and partial lack of efficacy (21), secondary loss of efficacy, intolerance, or other reasons). The reason of discontinuation was based on the opinion of the treating physician. Three treatment groups were formed according to the second biologic: RTX, anti-TNF mAb, and ETA. A sensitivity analysis gave similar results for the two biologics infliximab and adalimumab, and therefore these were merged together into one group (mAb).

The baseline characteristics of the three main groups were analysed by means of descriptive statistics. For normally distributed variables (given as mean \pm SD), an independent Student's *t*-test was used, while for the non-normally distributed variables [given as median (IQR)], the Mann-Whitney *U*-test was used. Fisher's exact test was used for comparison of categorical data. Difference between the groups regarding DAS28 improvements was investigated by an analysis of variance (ANOVA). An analysis of covariance (ANCOVA) was performed for correction of baseline differences between groups. All statistical tests were evaluated at a 0.05 significance

level. *P*-values and 95% confidence intervals (CIs) are presented. The statistical analysis was performed with SPSS version 20.

Results

A total of 328 patients who previously failed one TNF inhibitor were identified. Of these, 259 switched to another TNF inhibitor (161 received an anti-TNF mAb as a second biologic and 98 received treatment with ETA) and 69 switched to RTX.

Baseline characteristics

The baseline characteristics for patients in the three groups are summarized in Table 1. Significant differences were found between the groups regarding age, rheumatoid factor (RF) positivity, baseline HAQ score, number of SJC, and CRP. Patients who switched to RTX had higher disease activity (as assessed with DAS28, HAQ, SJC, and CRP) at baseline, mainly compared to mAb.

Effectiveness of treatment

The course of treatment was followed during the first 6 months. There was a significant improvement in DAS28 for both RTX and anti-TNF groups from baseline to 6 months (paired *t*-test, $p < 0.0001$ for all groups) (Table 2). The mean (\pm SD) improvement in DAS28 was significantly lower for mAb vs. RTX and ETA, and the difference remained statistically significant after correction for baseline differences (age, RF, baseline DAS28, and HAQ) (Table 2). In Table 2 changes at 6 months in DAS28 components as well as CRP and HAQ are also included. At the end of 6 months the proportions of patients who achieved a EULAR Good/Moderate/No response were: 22.9/54.2/22.9% for RTX (total number of patients with available information $n = 35$), 33.3/33.3/33.4% for ETA ($n = 48$), and 13.8/29.9/56.3% for mAb-treated

Table 1. Baseline demographics, disease and treatment characteristics of patients who switched to rituximab (RTX), a monoclonal antibody (mAb), or etanercept (ETA) after the failure of one TNF inhibitor.

	anti-TNF mAb [161]	ETA [98]	RTX [69]	<i>p</i> -value
Age (years), mean \pm SD	55.8 \pm 13.8 [161]	52.7 \pm 14.4 [98]	60.3 \pm 14.0 [69]	0.02 (mAb vs. RTX) 0.001 (ETA vs. RTX)
Sex (% female)	78.9 [161]	87.8 [98]	84.1 [69]	NS
Disease duration (years), median (IQR)	6 (3–15) [160]	7 (2–15) [98]	9 (3–16) [69]	NS
RF (% positive)	78.9 [161]	77.6 [98]	91.3 [69]	0.05 mAb and ETA vs. RTX
Pain VAS, mean \pm SD	52.5 \pm 24.8 [133]	51.4 \pm 25.3 [79]	58.4 \pm 20.6 [51]	NS
DMARDs (%)	68.9 [161]	71.4 [98]	59.4 [69]	NS
MTX (%)	64.0 [161]	61.2 [98]	42.0 [69]	NS
Corticosteroids (%)	45.3 [161]	54.1 [98]	58.0 [69]	NS

RF, Rheumatoid factor; VAS, visual analogue scale; DMARD, disease-modifying anti-rheumatic drug; MTX, methotrexate. The numbers of patients under study are given in square brackets.

Table 2. Effectiveness of rituximab (RTX), anti-TNF monoclonal antibody (mAb), and etanercept (ETA) after the failure of one TNF inhibitor during the first 6 months of therapy, according to DAS28 status and improvement, HAQ status and improvement, and changes in DAS28 components.

	anti-TNF mAb	ETA	RTX	Uncorrected p-value	Corrected p-value for age, RF, baseline DAS28, and HAQ
DAS28 baseline	4.87 ± 1.27 [120]	4.86 ± 1.21 [73]	5.30 ± 1.29 [43]	0.06 mAb vs. RTX	
DAS28 6 months	3.95 ± 1.34 [103]	3.99 ± 1.27 [62]	3.81 ± 0.98 [51]	< 0.0001 mAb vs. RTX	
Improvement in DAS28 0–6 months	0.67 ± 1.36 [83]	1.40 ± 1.51 [47]	1.70 ± 1.18 [34]	< 0.0001 mAb vs. RTX	0.006 mAb vs. ETA
HAQ baseline	1.14 ± 0.65 [126]	1.14 ± 0.62 [75]	1.43 ± 0.57 [47]	0.007 (mAb vs. RTX)	0.01 (ETA vs. RTX)
HAQ 6 months	0.97 ± 0.63 [102]	0.99 ± 0.7 [65]	1.24 ± 0.8 [47]	0.03 (mAb vs. RTX)	0.08 (ETA vs. RTX)
Improvement in HAQ 0–6 months	0.07 ± 0.51 [87]	0.23 ± 0.41 [47]	0.16 ± 0.54 [37]	NS	NS
SJC baseline	5 (2.25–9) [140]	6 (3–9) [82]	7.5 (4–11) [50]	0.03 (mAb vs. RTX)	0.07 (ETA vs. RTX)
SJC 6 months	2 (1–5) [110]	1 (0–3) [68]	2 (0–4.75) [56]	0.02 (mAb vs. RTX)	0.07 (ETA vs. RTX)
TJC baseline	6 (3–10) [140]	6 (2.75–9) [82]	5 (3–10) [51]	NS	NS
TJC 6 months	2 (1–6) [110]	1 (0–5) [68]	2 (1–4) [56]	NS	NS
PGA baseline	55.2 ± 24.0 [135]	53.75 ± 24.9 [79]	61.2 ± 20.1 [50]	0.08 (ETA vs. RTX)	
PGA 6 months	43.25 ± 26.1 [109]	39.6 ± 25.3 [67]	44.36 ± 25.9 [53]	NS	
ESR baseline	21 (11–36.5) [137]	20 (13–39) [83]	27 (14.75–48.25) [54]	0.05 (mAb vs. RTX)	
ESR 6 months	11 (11–31) [107]	17 (9–26) [65]	18 (12.75–26) [54]	0.03 (mAb vs. RTX)	
CRP baseline	7 (2–22.75) [144]	7 (2–22.5) [85]	15 (4.25–37.5) [56]	0.004 (mAb vs. RTX)	0.014 (ETA vs. RTX)
CRP 6 months	5 (1–14) [105]	4 (1–12) [66]	4 (2–11) [56]	0.003 (mAb vs. RTX)	0.016 (ETA vs. RTX)

DAS28, Disease activity score based on 28 joints; HAQ, Health Assessment Questionnaire; SJC, swollen joint count; TJC, tender joint count; PGA, patient's global assessment; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.

Values for normally distributed variables are given as mean ± SD, those for non-normally distributed variables as median (IQR).

The numbers of patients with available information are given in square brackets.

Table 3. Number of patients who failed one TNF inhibitor (mAb or ETA) and switched to an alternative TNF inhibitor (mAb or ETA) or RTX, according to the type of TNF switch.

Second biologic	First TNF inhibitor		Total
	mAb	ETA	
mAb	24	137	161
ETA	98	NA	98
RTX	35	34	69
Total	157	171	328

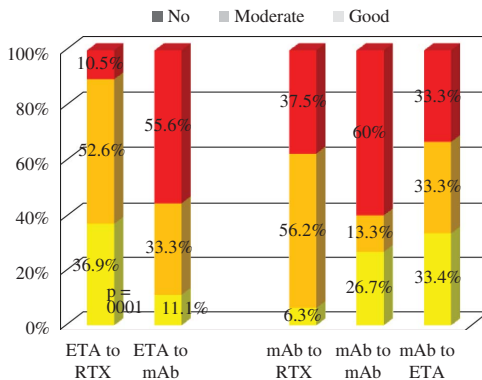


Figure 1. EULAR Good, Moderate, and No response for patients treated with RTX, mAb, or ETA according to the type of switch (whether they switched from mAb or ETA). After the failure of ETA, a significantly greater EULAR Good response rate was observed for RTX (36.9%) compared to mAb (11.1%); $p = 0.001$. After the failure of mAb, RTX and ETA yielded similar EULAR Good/Moderate response rates (no statistical difference was observed) that were numerically higher than mAb.

patients ($n = 87$). The difference in Good vs. No responders was statistically significant between ETA and mAb ($p = 0.003$) and between RTX and mAb ($p = 0.02$).

Effectiveness of switching according to the type of TNF switch

We analysed the effectiveness of RTX and anti-TNF according to the type of TNF switch. Table 3 shows the number of patients who switched from mAb and ETA to mAb, ETA, or RTX. Having failed ETA, RTX was significantly better than mAb [improvement in DAS28 0–6 months = 1.96 ± 0.90 ($n = 18$) vs. 0.69 ± 1.34 ($n = 68$), $p = 0.01$, 95% CI 0.10–0.75, p corrected for age, RF, baseline DAS28, and HAQ]. For patients who had failed mAb, RTX and ETA led to similar improvements in DAS28 [improvement in DAS28 0–6 months = 1.42 ± 1.40 ($n = 16$) and 1.40 ± 1.51 ($n = 47$), respectively]. These improvements were numerically higher than for TNF mAb [improvement in DAS28 0–6 months = 0.55 ± 1.53 ($n = 15$)].

Similar results were observed when we examined the effectiveness based on the percentages of EULAR Good/Moderate/No responders (Figure 1).

Reasons for discontinuation of previous treatment

In general, when the reason for discontinuation of the first TNF inhibitor was inefficacy, more patients switched to a second TNF inhibitor (either mAb or ETA) than RTX (57.9% and 31.9%, respectively, $p < 0.0001$) (Table 4). The opposite was seen for the group of patients who experienced an adverse event during the first anti-TNF therapy. A greater percentage of patients switched then to RTX (43.5%) than a second anti-TNF (19.7%) (Table 4).

The reason for discontinuation of the previous treatment appeared to be related to the efficacy of the next treatment. When the cause for switching was intolerance, RTX achieved significantly greater improvement in DAS28 ($p = 0.02$, 95% CI 0.08–0.89) and significantly greater EULAR Good/Moderate response rates ($p = 0.04$) compared to anti-TNF mAb (Table 4). RTX-treated patients achieved numerically greater DAS28

Table 4. Number and effectiveness of the second biologic (RTX, anti-TNF mAb, and ETA) after failure of one TNF inhibitor according to the reason for discontinuation of the first biologic (inefficacy, primary or secondary, intolerance, or other reasons, including pregnancy, patient’s decision, unknown reasons).

Reason for discontinuation of the first TNF inhibitor	RTX [$n = 69$]	anti-TNF mAb [$n = 161$]	ETA [$n = 98$]
Inefficacy, n (%)	22 (31.9)	97 (60.3)	53 (54.1)
Improvement in DAS28 0–6 months, mean \pm SD	1.22 ± 1.52 [10]	0.70 ± 1.41 [45]	1.54 ± 1.43 [30]
EULAR Good/Moderate/No responders (n)	1/5/4 (10)	6/13/28 (47)	9/12/9 (30)
Primary inefficacy, n (%)	9 (13.0)	47 (29.2)	33 (33.7)
Improvement in DAS28 0–6 months, mean \pm SD	-0.18 ± 0.83 [4]	0.64 ± 1.51 [22]	1.76 ± 1.20 [19]
EULAR Good/Moderate/No responders (n)	0/0/4 (4)	2/6/16 (24)	5/10/4 (19)
Secondary inefficacy, n (%)	13 (18.9)	50 (31.1)	20 (20.4)
Improvement in DAS28 0–6 months, mean \pm SD	2.16 ± 1.05 [6]	0.75 ± 1.34 [23]	1.18 ± 1.76 [11]
EULAR Good/Moderate/No responders (n)	1/5/0 (6)	4/7/12 (23)	4/2/5 (11)
Intolerance, n (%)	30 (43.5)	26 (16.1)	25 (25.5)
Improvement in DAS28 0–6 months, mean \pm SD	2.09 ± 0.87 [16]	0.63 ± 1.44 [11]	0.91 ± 1.62 [10]
EULAR Good/Moderate/No responders (n)	5/9/3 (17)	2/4/7 (13)	4/1/5 (10)
Other, n (%)	17 (24.6)	38 (23.6)	20 (20.4)
Improvement in DAS28 0–6 months, mean \pm SD	1.55 ± 1.12 [8]	0.63 ± 1.30 [27]	1.44 ± 1.75 [7]
EULAR Good/Moderate/No responders (n)	2/5/1 (8)	4/7/14 (25)	3/3/1 (7)

improvements ($p = 0.3$, 95% CI 0.52–1.60) and EULAR Good/Moderate responses ($p = 0.09$) compared to ETA.

When the cause for switching was ineffectiveness, the improvement in DAS28 was similar for RTX, ETA, and mAb. The EULAR Good/Moderate responders were 60, 70, and 40.4% for RTX, ETA, and mAb, respectively ($p = 0.05$ between RTX and mAb, $p = 0.02$ between mAb and ETA). However, after a closer examination of the type of ineffectiveness of the prior TNF inhibitor, we observed that, after loss of efficacy (secondary inefficacy) to a TNF inhibitor, RTX achieved significantly greater DAS28 improvements than mAb ($p = 0.03$, 95% CI 0.26–2.55) as well as better EULAR response rates ($p = 0.03$). Numerically but not significantly greater results were observed for RTX vs. ETA. No significant difference in DAS improvement or EULAR response between the groups was observed after primary inefficacy of the first TNF inhibitor, and the number of patients was fairly small. The p -values shown were corrected for age, RF, baseline DAS28, and HAQ.

Discussion

The results of this observational cohort suggest that, after the failure of one TNF inhibitor, a treatment approach with the B-cell depleting agent RTX leads to better overall results, based on DAS28 improvement and EULAR Good/Moderate response, than a second TNF inhibitor. However, a significant difference was observed only between RTX and anti-TNF mAb and not between RTX and ETA. The absence of a significant difference might be due to a true difference (if we also consider the molecular differences in etanercept and monoclonal antibodies), but it could also be due to insufficient power. In all three groups (mAb, ETA, and RTX), significant DAS28 improvements were observed, suggesting that switching from one anti-TNF to another is also effective.

When we compared the efficacy of RTX to the efficacy of anti-TNF by the type of anti-TNF switch, we observed the smallest improvements in DAS28 for the subgroup of patients who switched from one anti-TNF mAb to an alternative mAb. By contrast, after the failure of a first mAb, ETA yields as good results as RTX (Figure 1). Although statistical significance was not achieved, this finding might suggest that, after a failure of one TNF mAb, ETA or RTX could be a better choice as the next treatment than an alternative mAb. Previous studies have also shown greater benefits when switching between ETA and mAb (22). The greatest reduction in DAS28 was observed in patients treated with RTX after having failed ETA, which was significantly greater than the observed DAS28 reduction for patients who switched to mAb. Significantly better EULAR responses were also observed (Figure 1).

Regarding the reason for discontinuation of a prior TNF inhibitor, our results do not agree with those of a large observational study from Switzerland (23), in which Finckh et al showed that patients who stopped a previous TNF inhibition treatment due to ineffectiveness had better results when they switched to RTX rather than an

alternative TNF inhibitor. By contrast, we unexpectedly observed better results of treatment with RTX when the reason for discontinuation of a previous anti-TNF agent was intolerance and secondary inefficacy. In Stockholm, most patients fail at least two TNF antagonists before switching to a different biological agent. Ineffectiveness of a previous treatment could more frequently be the motive to switch from a TNF mAb to ETA, rather than to switch to another category of biologics, as shown in Table 4. We should take into consideration the fact that the choice of the biologic agent is not random, as it is the individual rheumatologist who decides based on the patient's clinical assessment and according to the hospital's protocols, and these factors of course vary between countries and even within a particular country. The risk for selection bias is therefore present. In addition, the reason for discontinuation of a prior biologic given by a significant number of patients was neither lack of efficacy nor intolerance, but 'other'. This could be the patient's own decision, pregnancy, or an unknown reason. Importantly, one such reason could also be 'failure by partial efficacy', which can in fact represent a substantial percentage of patients who have neither an outstanding response nor a completely lack of response to therapy (21). This will be examined further in future analyses.

One weakness of this observational study was the fact that the three main treatment groups were not balanced for baseline characteristics, and differences in several baseline characteristics were observed. Patients who switched to RTX had higher disease activity at baseline (based on HAQ, DAS28, SJC, and CRP), and therefore a greater improvement in disease activity markers could be explained, at least in part, by regression to the mean. Nevertheless, the majority of RTX-treated patients were patients with high inflammatory activity and longstanding, difficult to control RA, who had failed to respond to previous therapies, and might be considered to have more refractory, 'hard-to-treat' disease. To partially overcome this problem we adjusted for baseline differences when comparing treatment effect between the groups and subgroups. The number of missing data was another concern, but it is a common problem in observational cohorts. We used LOCF for DAS28 at 6 months to increase the number of patients with available information, but the number of patients in some comparisons was still fairly small (especially in the subanalysis of 'reason of discontinuation'), so we have to interpret some results with caution.

Among the strengths of this study is the fact that we investigated real-life patients with demographic, serological, and disease-related characteristics consistent with populations seen in clinical praxis. The number of patients is reasonably large, and they represent a relatively homogeneous population. We chose to examine the efficacy of only the first switch, and not merge switches together to avoid the bias of 'common patients' history' in the cohort.

In conclusion, for patients who failed one TNF inhibitor, RTX leads to better clinical results than a subsequent anti-TNF mAb. Having failed ETA, RTX is better than

mAb, but having failed mAb, RTX and ETA yield similar improvements in disease activity. In this cohort, patients who previously did not tolerate or who lost efficacy of the first TNF inhibitor achieved better results with RTX.

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Effectiveness and survival-on-drug of certolizumab pegol in clinical practice – results from the national Swedish register

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Abstract

Objectives: Evidence regarding the efficacy and effectiveness of certolizumab pegol in RA patients who have already failed a TNF inhibitor is limited. The aim of this study was to describe the effectiveness and survival-on-drug of certolizumab pegol in a real-life setting, both in TNF-inhibitor (TNFi)-naïve patients and in patients who had failed previously TNFis, and in relation to disease activity at baseline.

Methods: The national Swedish Rheumatology Quality of Care Register was used to identify patients with RA starting treatment with certolizumab pegol 2009 through 2013. Effectiveness of treatment was assessed using DAS28, HAQ, measures of remission, EULAR response and survival-on drug through 0-8 months from treatment start.

Results: A total of 945 RA patients started treatment with certolizumab pegol. 540 patients (57.1%) received certolizumab as 1st biologic treatment, 215 patients (23%) had failed one previous TNFi and 190 (20%) had failed at least two TNFis. Overall 71% achieved at least a EULAR Moderate Response and 38% had a EULAR Good Response at 6 months from baseline. TNFi-naïve patients achieved significantly better results and had better survival-on-drug compared to patients who had failed previous TNFis. Around 20% of patients who had not responded to two or more prior TNFis achieved EULAR good response to therapy and around 20% achieved remission. Patients who had high baseline disease activity had higher risk to discontinue treatment compared to those with non-high disease activity.

Conclusions: In this real-life RA cohort, certolizumab pegol was associated with significant clinical improvement. The effectiveness and survival-on-drug varies depending on line of treatment.

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Introduction

Certolizumab pegol is one of five tumor necrosis factor inhibitors (TNFis) approved for the treatment of RA. It is a recombinant antigen-binding fragment (Fab') of a humanized antibody against TNF, conjugated to a polyethylene glycol (PEG) moiety [1 2]. Its efficacy and acceptable safety profile has been demonstrated in phase III, randomized, controlled clinical trials in methotrexate non-responders with RA of high disease activity [3-7].

So far, only one study has examined the efficacy of certolizumab pegol also in patients with moderate disease activity[8]. However, since more than half of patients in contemporary clinical practice do not have high disease activity at the time of start or switch of biologic therapy, it is important to examine the effectiveness of treatment in patients with moderate or even low disease activity at baseline[9].

Additionally, only one study has examined the efficacy of certolizumab pegol after the failure of one or more TNFis[10]. Among patients starting a first TNFi, half will discontinue treatment during the first two years of treatment because of inefficacy or intolerance[11 12]. Starting a second or more TNFi is common in clinical practice, and it is important to know if certolizumab pegol is effective in TNF-non-responders. Its different molecular structure makes it theoretically possible that it can be effective even after the failure of previous TNF inhibition with other agents. Furthermore, randomized controlled trials often have strict inclusion and exclusion criteria that do not reflect clinical practice[13 14]. This is the reason why register-based observational studies can provide important additional information about the effectiveness of treatment in a real-life RA population of patients.

The aim of this study was 1) to describe the real-life RA population that receives? treatment with certolizumab pegol; 2) to assess the effectiveness of treatment and survival-on-drug in TNFi naïve patients as well as in patients who have previously failed one TNFi and two or more TNFis; and 3) to examine the effectiveness of treatment and survival-on-drug in patients with high versus non-high disease activity at baseline.

Methods

Study population

Data from the nationwide Swedish Rheumatology Register were used. RA patients who start treatment with biologic agents have been included in this register since 1999. The coverage of the SRQ in terms of biologics-treated RA has been estimated to be nearly 90% of all eligible patients with RA[15 16]. The majority of RA patients in Sweden start a biologic treatment (most often an anti-TNF agent unless contraindicated) after failure of at least one synthetic DMARD, like methotrexate.

For the purpose of this study patients with a diagnosis of RA who started treatment with certolizumab pegol during the period 01 October 2009 – 31 June 2013 were identified and selected. The last observation date was 01 March 2014, so all patients could contribute with follow-up data. Four different reasons for discontinuation of certolizumab were considered:

primary inefficacy (lack of efficacy, including partial efficacy), secondary inefficacy (loss of efficacy), intolerance and other (pregnancy, patient's or physician's decision, inactive disease, death, unknown). The reason for discontinuation was based on the rheumatologist's opinion and was recorded according to a predefined list of different options.

The following information was collected: demographic data (age, sex); RA disease duration (from the time of diagnosis); rheumatoid factor; number and type of prior biologics, date and reason for discontinuation of certolizumab pegol; disease activity score based on 28-joint status (DAS28) and its components (swollen joint count, tender joint count, visual analogue scale general health and erythrocyte sedimentation rate); functional ability based on health assessment questionnaire (HAQ); concomitant DMARD and glucocorticoid treatment.

Effectiveness of treatment was assessed as: 1) DAS28 change (Δ DAS28) and HAQ change (Δ HAQ), 2) Proportion of patients in state of DAS28-remission, 3) EULAR response rates and 4) Survival-on-drug.

The endpoints 1-3 were assessed at 3 and 6 months from baseline. Baseline was defined as start of certolizumab pegol. The time window was 30-110 days from baseline for the 3-month visit and 110-240 days from baseline for the 6-month visit. In case of multiple visits within a visit-window we used the first observation chronologically. When no data were available for the 6-month visit we used data from the 3-month visit. Patients who discontinued treatment during the follow-up period but had no follow-up visit were considered non-responders. The dataset was not complete. Non-completeness is expected in register-based observational studies. We explored missingness and the summary is reported in figure 1.

Statistical analysis

Initially we characterized the whole cohort by means of descriptive statistics. At a second step three subgroups were formed based on the number of prior biologic TNFi: 0 (TNFi-naïve patients), 1 (maximum of one prior TNFi) and ≥ 2 (two or more prior TNFi). An additional subanalysis was performed with patients stratified according to baseline DAS28 disease activity state: high (defined as $\text{DAS28} > 5.1$) versus other ($\text{DAS28} \leq 5.1$). Baseline characteristics across the groups were summarized and compared. The normality of all continuous variables was tested by assessing the skewness. Variables which were similar to normal distribution were presented as mean \pm SD, while non-normally distributed variables were presented as median with IQR. Analysis of variance (ANOVA) was used to compare continuous variables (with a distribution similar to normal) followed by Bonferroni test for post hoc comparisons between the groups when the ANOVA showed significant difference across the groups, while χ^2 test was used for nominal variables. Kruskal-Wallis test was used to compare non-normally distributed variables. The level of statistical significance was set to 5%.

The mean DAS28, Δ DAS28, HAQ and Δ HAQ at each time-point were compared across the groups by ANOVA followed by Bonferroni test for post hoc comparisons between the groups.

The rates of EULAR good responders and patients that achieve DAS28 remission were compared by χ^2 test. All analyses were performed for the whole cohort and stratified by number of TNFis previously discontinued for various reasons (ineffectiveness, intolerance, other) and by baseline disease activity state.

A Cox regression analysis was performed to determine the risk for discontinuation of certolizumab pegol during the first 30 months according to the number of prior TNFis and according to the disease activity state at baseline (two separate analyses). Both analyses were adjusted for age, sex, disease duration and concomitant DMARDs. Kaplan-Meier plots were designed. Discontinuation of treatment and death were considered events. Continuation of treatment during follow-up was treated as censored observations during the analysis. Patients who had no follow-up visit were also censored.

Results

Baseline characteristics

A total of 945 RA patients started treatment with certolizumab pegol during the study period. Baseline characteristics (baseline=start of treatment) are presented in table 1. Patients had long disease duration when they started treatment (median duration 9.1 years). The majority was RF positive and received concomitant treatment with a synthetic DMARD. Half of patients were treated with glucocorticoids.

Stratification according to the number of prior TNFis and disease activity state at baseline

540 patients (57.1%) received certolizumab as 1st biologic treatment, 215 patients (23%) had tested 1 previous TNFi and 190 (20%) had tested at least 2 TNFis. Baseline characteristics for patients according to the number of prior TNFis are shown in table 1. Patients who had failed at least 2 prior TNFis had significantly longer disease duration, higher disease activity and more functional disability at baseline compared to TNFi-naïve patients.

Out of 753 patients with available DAS28 information at baseline, 292 (39%) had high disease activity (DAS28>5.1) while 461 (61%) had lower disease activity. Patients with high disease activity were significantly older compared to those with lower disease activity (mean age \pm SD in years = 58.6 ± 13.7 vs. 55.3 ± 13.6 , respectively), had significantly higher HAQ at baseline (mean HAQ \pm SD = 1.4 ± 0.6 vs. 0.9 ± 0.6 , $p<0.0001$) and were less likely to be treated with concomitant DMARDs (62% vs. 70%, $p=0.02$). The two groups did not differ significantly with regard to disease duration, sex, RF and concomitant corticosteroids.

Effectiveness of treatment

In table 2 the mean DAS28, Δ DAS28, HAQ and Δ HAQ at baseline, 3 and 6 months for all patients and after stratification according to the prior TNFis group is shown. Significant reductions in DAS28 and HAQ were achieved for the whole cohort of patients and for each group separately (table 2). DAS28 and HAQ were significantly lower in TNFi-naïve patients

compared to patients with 1 and ≥ 2 prior TNFis at 3- and 6 month-visit. TNFi-naïve patients also achieved significantly greater DeltaDAS28 compared to both other two groups (table 2).

The proportion of patients in remission at 6 months for patients with 0, 1 and ≥ 2 prior TNFis was 42%, 26% and 18%, respectively (the difference being statistically significant between all three groups, $p<0.0001$). Overall 33%/28%/39% achieved EULAR good/moderate/no response at 6 months from baseline. The proportion of EULAR good responders was significantly greater for patients with 0 prior TNFis compared to those with 1 and ≥ 2 prior TNFis at 3 and 6 months (figure 2).

At 6 months patients with high disease activity at baseline achieved significantly greater DeltaDAS28 compared to those with lower disease activity at baseline (-2.1 ± 1.4 vs. -1.0 ± 1.4 , respectively, $p<0.0001$), as expected. Conversely, patients with non-high disease activity achieved remission to a significantly greater extent compared to those with high disease activity initially (43% vs. 21%, $p<0.0001$). EULAR Good response rates were similar between the two groups (35% and 34%, $p=0.6$).

Survival-on-drug

During the observation period 365 patients (39%) discontinued treatment. The reason for discontinuation was primary inefficacy (N=159), secondary inefficacy (N=62), intolerance (N=86) and other reasons such as patient's decision, pregnancy and others (N=58). The number of patients discontinuing treatment was positively correlated to the number of prior TNFis (30% in TNFi naïve patients, 54% in patients having failed 1 prior TNFi and 46% in those having failed 2 or more TNFis).

The survival-on-drug curve stratified by number of prior TNFis group is shown in figure 3A. In the adjusted Cox regression analysis patients with 1 and ≥ 2 prior TNFis had significantly higher risk of discontinuing treatment compared to TNFi naïve patients [HR=1.4 (95% CI: 1.1-1.9), $p=0.004$ and HR=1.7 (95% CI: 1.3-2.2), $p<0.0001$, respectively]. In the regression model the use of concomitant DMARDs at start of certolizumab pegol treatment was also associated with significantly better survival-on-drug [HR=1.4 (95% CI: 1.1-1.7), $p=0.003$]. In figure 3B survival-on-drug by baseline disease activity state (high vs. non-high) is shown. Patients with non-high disease activity at the time of certolizumab pegol start had significantly lower risk to discontinue treatment compared to those with high disease activity [HR=0.7 (95% CI: 0.6-0.9), $p=0.02$].

Discussion

The results of this observational study supports the findings of double-blind, placebo-controlled trials, that certolizumab pegol is effective for patients with active RA. Significant clinical improvements were observed during the first 8 months of treatment as assessed by DAS28 reduction, remission rates and EULAR responses. The survival-on-drug was similar to other TNFis as demonstrated in other observational studies[17 18].

This large national cohort included patients with long-standing RA (median disease duration 9 years). Even patients who were TNFi naïve had a median of disease duration of 6 years (table

2). More than half of all patients in the cohort were TNFi naïve. We differentiated previous TNFis used from previous biologics used in order to examine the effectiveness of certolizumab pegol after the failure of TNF inhibition. Only a small percentage (13 out of 540) of TNFi-naïve patients had tested another biologic (rituximab, tocilizumab, anakinra or abatacept) before certolizumab pegol.

It has been shown from previous studies that switching to a second or a third TNFi after inadequate response to one or two TNFis might still yield clinically significant results[12 19 20]. This is most likely due to the fact that there are differences in the molecular structure, pharmacodynamics and pharmacokinetics that differentiate the TNFis that are available today, even though they target the same cytokine. In this study, patients who were naïve to TNF inhibition achieved significantly greater DAS28 reduction at 3 and 6 months from baseline compared to those having failed one and those having failed two or more prior TNFis (table 2). We also observed a further increase of the absolute magnitude of the DeltaDAS28 from 3 to 6 months for the group 0 and 1, while DeltaDAS28 decreased in group ≥ 2 (table 2). The proportion of patients in remission and who achieved EULAR good response was significantly greater for TNF naïve patients compared to both other groups (figure 2). This finding was expected and is in agreement with previous findings from observational studies showing inferior response rates when switching to a second or third TNFi[19 20]. An interesting and important observation was that even after having previously received 2 or more TNFis 55% of patients can achieve EULAR good or moderate response and around 20% can achieve remission.

These results are partially in agreement with the results from the REALISTIC study, where the efficacy (defined as ACR20 response) of certolizumab pegol was demonstrated even for patients with previous TNFi use, regardless of the number or type of previous TNFi used[10]. In that trial TNF naïve patients achieved higher ACR 50 and 70 responses compared to patients with previous TNFi use (29.6% vs. 21.6% and 15.3% vs. 9.1%, respectively). However the trial was not designed to detect significant differences between these groups.

In this study more than half of patients had low or moderate EULAR disease activity based on DAS28 at the time of certolizumab pegol initiation, reflecting the real-life character of the cohort. Clinically and statistically significant responses were observed regardless of whether the initial disease activity state was high or not. Patients with high disease activity initially were less likely to achieve remission and to continue with the treatment compared to other patients. These results are in agreement with the CERTAIN trial, where low-disease activity or remission was reached by the majority of patients who received certolizumab pegol and who had predominantly moderate disease activity at baseline[8].

The use of concomitant DMARDs was associated with longer survival-on-drug in the Cox regression analysis. Previous studies have shown that concomitant DMARD treatment is associated with better response to treatment with TNFis[21 22]. Since significant difference in concomitant DMARD rates was found across the prior TNFis groups (table 1), it was important to include concomitant DMARDs in the Cox regression analysis in order to

minimize risk for confounding when comparing the risk for discontinuing treatment according to the number of prior TNFis.

The observational character of this study is connected to certain limitations. Baseline characteristics between groups under comparison were not completely balanced and significant differences were observed which introduce the risk for confounding. We tried to partially overcome this problem by adjusting for variables that differed significantly between groups in the Cox regression analysis. Missingness is another common problem in register-based observational studies. In this study 70% of patients who had available DAS28 at baseline had available DAS28 information at 6 months. As it is shown in figure 1, however, only 52 patients out of 753 patients with baseline DAS28 information (7%) were lost to follow up. On the other hand there are significant strengths, such as the large number of patients included in the cohort, the opportunity to examine the effectiveness of treatment in a TNFi naïve population and in patients who have already failed one or more TNFis, as well as in patients with moderate or even low disease activity at baseline. This is the first observational study to our knowledge that has examined the effectiveness of certolizumab pegol in this context.

To conclude, certolizumab pegol is effective in a real-life RA population. Response rates are highest and survival-on-drug best when given as first-line TNFi, but can lead to significant clinical results even after the failure of one or more TNFis. Patients with non-high disease activity at baseline were more likely to achieve remission and to continue with the treatment compared to those with high activity.

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Variable	All patients [945]	Number of prior TNF inhibitors			Difference between groups (p-value)
		0 [540]	1 [215]	≥2 [190]	
Age (years)	56.4 ± 13.8 [945]	55.7 ± 13.9 [540]	57.7 ± 13.7 [215]	57.1 ± 13.6 [190]	
Sex (% female)	75.2% [945]	72.2% [540]	75.3% [215]	83.7% [190]	0 vs. ≥2: 0.002 1 vs. ≥2: 0.04
Disease Duration (years)	9.1 (3.6-17.7) [937]	6 (2-12.8) [535]	10.9 (5.6-18.9) [213]	15 (9.9-23.7) [188]	<0.0001 between all groups pairwise
RF	79.5% [860]	78.1% [506]	79.5% [185]	84.0% [169]	
Conc. DMARD (% yes)	65.4% [945]	70.2% [540]	63.7% [215]	53.7% [190]	0 vs. 1: 0.08 0 vs. ≥2: <0.0001 1 vs. ≥2: 0.04
Conc. Glucocorticoids (% yes)	49.6% [945]	49.8% [540]	44.7% [215]	54.7% [190]	
- Dose (mg)	6.3 (5-10)	5 (5-10)	7.5 (5-10)	7.5 (5-10)	

Table 1. Baseline characteristics for Swedish patients with RA starting treatment with certolizumab pegol in the SRQ register stratified by the number of prior TNF inhibitors (0, 1 or ≥2). Number in brackets indicates the number of patients with available information for each variable. One-way analysis of variance was used to compare normally distributed continuous variables followed by Bonferroni test for post hoc comparisons between the groups, while χ^2 test was used for nominal variables. Kruskal-Wallis test was used to compare non-normally distributed continuous variables across groups.

	Variable	All patients	Number of prior TNF inhibitors			Difference across groups (p-values)	Difference between groups (p-values)
			0 [540]	1 [215]	≥2 [190]		
DAS28	Baseline	4.6 ± 1.4 [753]	4.6 ± 1.4 [447]	4.6 ± 1.4 [159]	5.0 ± 1.5 [147]	0.01	0 vs. 2: 0.01 1 vs. 2: 0.04
	3 months	3.5 ± 1.5 [365]	3.1 ± 1.5 [218]	3.7 ± 1.3 [79]	4.2 ± 1.5 [68]	<0.0001	0 vs. 1: 0.008 0 vs. 2: <0.0001 0 vs. 1: 0.001
	6 months	3.3 ± 1.5 [513]	3.0 ± 1.4 [303]	3.5 ± 1.2 [110]	4.1 ± 1.6 [100]	<0.0001	0 vs. 2: <0.0001 1 vs. 2: 0.006
ΔDAS28	3 months	-1.3 ± 1.6 [321]	-1.5 ± 1.6 [197]	-1.0 ± 1.5 [67]	-0.9 ± 1.6 [57]	0.02	0 vs. 2: 0.05
	6 months	-1.4 ± 1.5 [440]	-1.7 ± 1.5 [267]	-1.2 ± 1.3 [89]	-0.8 ± 1.7 [84]	<0.0001	0 vs. 1: 0.006 0 vs. 2: <0.0001 1 vs. 2: 0.5
HAQ	Baseline	1.1 ± 0.7 [820]	1.0 ± 0.6 [474]	1.1 ± 0.6 [181]	1.4 ± 0.7 [165]	<0.0001	0 vs. 1: 0.06 0 vs. 2: <0.0001 1 vs. 2: 0.003
	3 months	0.9 ± 0.7 [421]	0.7 ± 0.7 [252]	1.0 ± 0.6 [96]	1.3 ± 0.7 [73]	<0.0001	0 vs. 1: 0.01 0 vs. 2: <0.0001 1 vs. 2: <0.0001
	6 months	0.9 ± 0.7 [520]	0.7 ± 0.6 [306]	1.0 ± 0.6 [117]	1.3 ± 0.6 [105]	<0.0001	0 vs. 1: 0.005 0 vs. 2: <0.0001 1 vs. 2: 0.002
ΔHAQ	3 months	-0.2 ± 0.5 [382]	-0.2 ± 0.5 [229]	-0.2 ± 0.4 [86]	-0.2 ± 0.5 [67]	N.S.	
	6 months	-0.2 ± 0.5 [472]	-0.2 ± 0.5 [273]	-0.2 ± 0.5 [100]	-0.1 ± 0.5 [93]	N.S.	

Table 2. DAS28 and HAQ status at baseline, 3 and 6 months as well as ΔDAS28 and ΔHAQ at 3 and 6 months from baseline for the whole cohort and stratified by the number of prior TNF inhibitors (0, 1 or ≥2). Number in brackets indicates the number of patients with available information at each time-point. One-way analysis of variance was used to compare responses across groups followed by Bonferroni test for post hoc comparisons between the groups. N.S.=non-significant

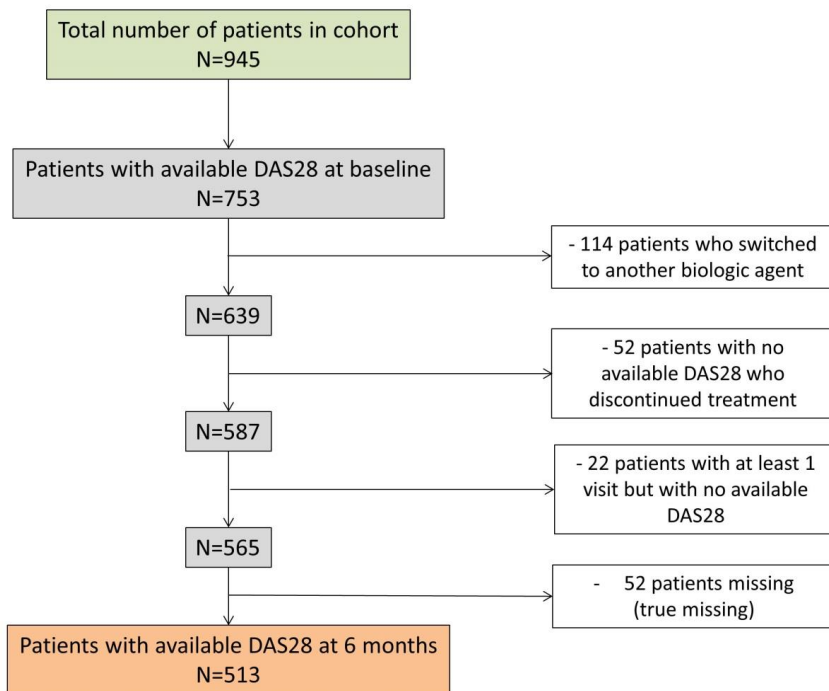


Figure 1. Type of missingness regarding DAS28. Out of 753 patients who had available DAS28 information at baseline 513 had available DAS28 at 6 month-visit (68%).

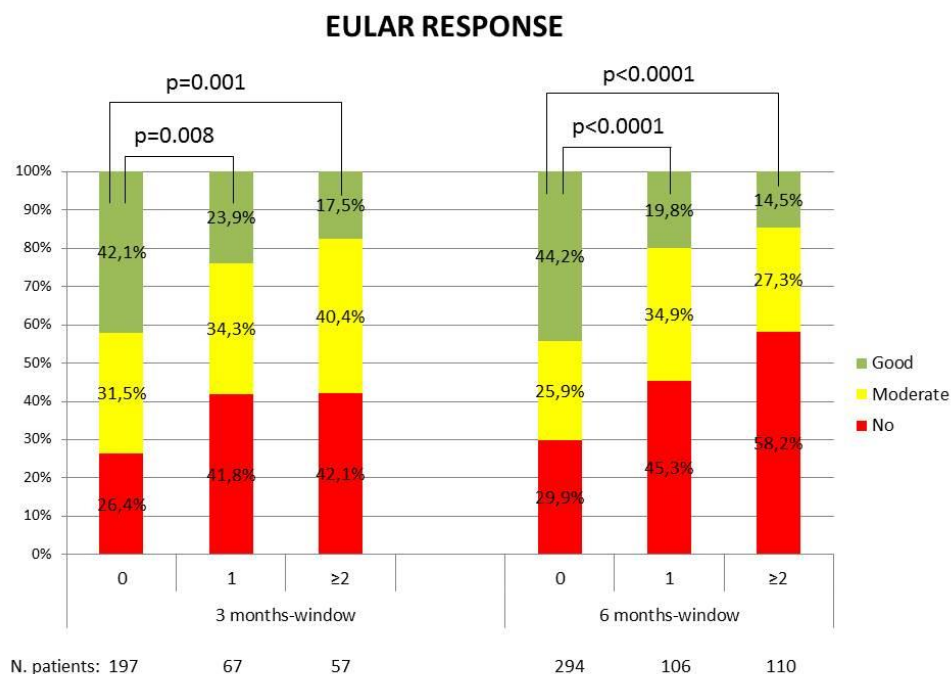
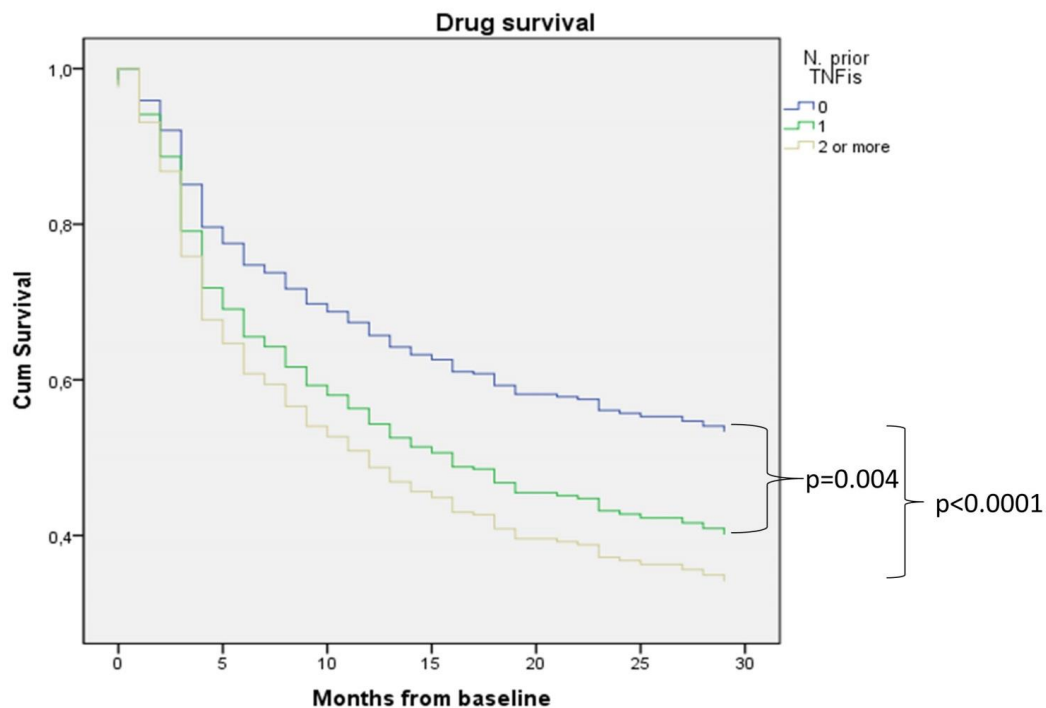


Figure 2. EULAR response at 3 and 6 months from baseline according to the number of prior TNFi (0, 1 and ≥ 2). Patients who were TNFi naïve (group 0) achieved significantly higher proportion of EULAR good responders at both time-points compared to those who had two or more prior TNFis (p-values shown). No significant differences were observed between groups 1 and 2. In the lowest line the number of patients with available information at each time point in each group is shown.



N. Patients	540	431	402	388	379	377	377
	215	156	136	125	119	117	116
	190	127	107	97	94	90	87

Figure 3A. Survival-on-drug during the first 30 months from baseline for certolizumab pegol stratified by the number of prior TNFis (0, 1 or ≥ 2) and adjusted for age, sex, disease duration and concomitant DMARDs in a Cox regression analysis. Patients with 1 and ≥ 2 prior TNFis had significantly higher risk to discontinue treatment compared to TNFi naïve patients [HR=1.4 (95% CI: 1.1-1.9), $p=0.004$ and HR=1.7 (95% CI: 1.3-2.2), $p<0.0001$, respectively]. At the bottom of the plot the number of patients who are on-treatment at each time-point and at each subgroup is shown (blue=0, green=1 and brown= ≥ 2 prior TNFis).

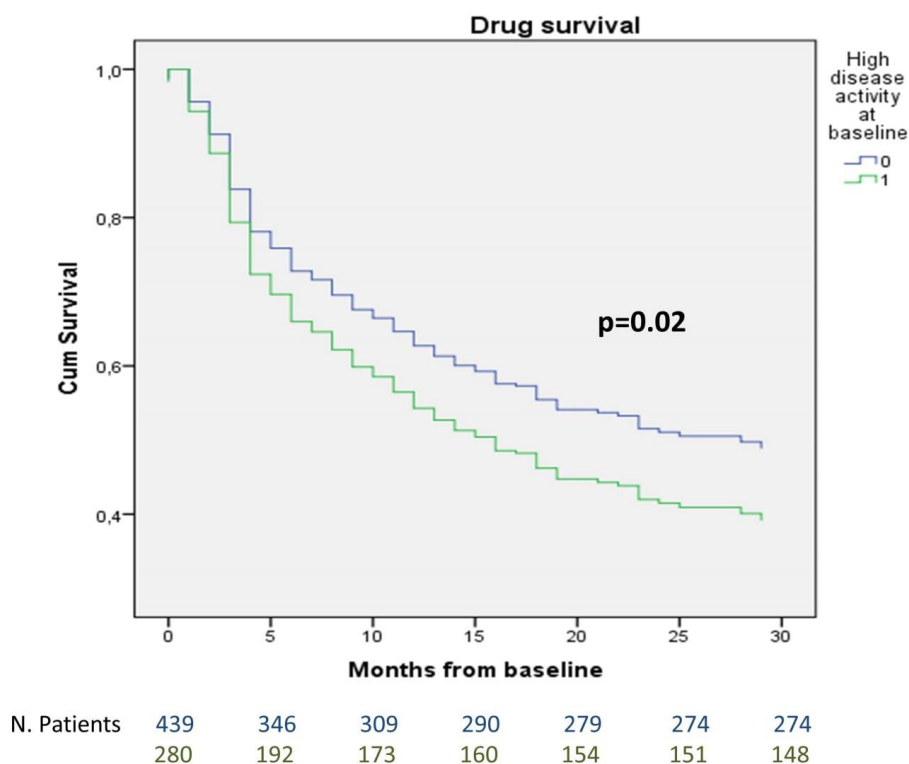


Figure 3B. Survival-on-drug during the first 30 months from baseline for certolizumab pegol stratified by the disease activity state at baseline (high=1 vs. non-high=0) and adjusted for age, sex, disease duration and concomitant DMARDs in a Cox regression analysis. Patients with non-high disease activity at the time of certolizumab pegol start had significantly lower risk to discontinue treatment compared to those with high disease activity [HR=0.7 (95% CI: 0.6-0.9), $p=0.02$]. At the bottom of the plot the number of patients who are on-treatment at each time-point and at each subgroup is shown (blue=no high disease activity, green= high disease activity at baseline).

VIII

A Multicenter, Randomized, Controlled, Open-Label Pilot Study of the Feasibility of Discontinuation of Adalimumab in Rheumatoid Arthritis Patients in Stable Clinical Remission

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Abstract

Background: Treatment with tumor necrosis factor (TNF) blockers, once started as therapy for rheumatoid arthritis (RA), is usually continued indefinitely.

Objective: To assess the possibility, in RA patients in stable remission on combination therapy with adalimumab (ADA) and methotrexate (MTX), of discontinuing ADA treatment while maintaining remission.

Methods: In a randomized, controlled, open pilot study of RA patients in stable remission treated with adalimumab + MTX patients were randomized in a 1:1 ratio to continue with adalimumab plus MTX (arm AM) or MTX monotherapy (arm M) for 52 weeks. Flare was defined as DAS28 \geq 2.6 or an increase in DAS28 (Δ DAS28) of more than 1.2 from baseline at any time. Patients in arm M with a flare restarted ADA. The primary endpoint was the proportion of patients in remission at week 28 in both arms.

Results: Thirty-three patients were enrolled in the study and were randomized to arm AM (16 patients) and arm M (15 patients). At 28 weeks, 15/16 patients (94%) and 5/15 patients (33%) in arms AM and M, respectively, were in remission ($p=0.001$). The proportion of patients with a flare during the first 28 weeks in the AM and M arms was 50% (8/16) and 80% (12/15), respectively ($p=0.08$). The number of patients in the AM and M arms with at least one Δ DAS28 >1.2 during first 28 weeks was 1/16 (6%) and 8/15 (53%), respectively ($p=0.005$).

Conclusions: In this study remission was rarely maintained in patients with long-standing disease on remission who discontinued adalimumab. Discontinuation may be feasible in only a minority of patients with established RA in stable clinical remission.

Introduction

The field of RA treatment has changed dramatically during the last decade. Better understanding of the pathophysiology and the underlying immunological mechanisms of the disease has led to tighter disease control, earlier treatment and the emergence of a new class of drugs, the biologic disease modifying antirheumatic drugs. TNF inhibitors were the first biologics to be approved for the treatment of severe RA. Adalimumab is a recombinant human immunoglobulin (IgG1) monoclonal antibody which binds with high affinity and specificity to TNF [1]. Its efficacy in RA and acceptable safety profile has been proven in large randomized, controlled clinical trials [2, 3].

The goal of treatment today is remission, clinical, functional and radiological. A further step is the sustained remission state without the need of continuous treatment with a biologic agent, the achievement of 'biologic-free' remission. If remission could be sustained even after cessation of anti-TNF therapy, this would have vast clinical (regarding long-term safety) as well as economic implications. Treatment with tumor necrosis factor (TNF) blockers, once started as therapy for rheumatoid arthritis (RA), is usually continued indefinitely. This is mainly due to the fact that information about the feasibility to discontinue anti-TNF therapy in RA patients who have obtained remission is limited. In the ATTRACT study, RA patients with longstanding disease received treatment with infliximab, and in 17 patients this was discontinued after 2 years [4]. All 17 patients experienced a flare. In contrast, in a study of patients with early RA, 70% of those initially treated with infliximab could discontinue the TNF inhibitor while remaining in remission [5]. In the RRR study by Tanaka et al., of 102 patients 56 (55%) maintained low disease activity and 44 (43%) fulfilled the criteria for clinical remission 1 year after discontinuation of infliximab [6]. The mean disease duration in this study was 6 years, suggesting that discontinuation of the TNF inhibitor might be feasible not only in patients with early RA, but also in those with established, long standing disease.

Apart from the duration of RA, other factors can influence the chance of biologic-free remission, such as the time from diagnosis to anti-TNF introduction. In the BeST study, it was shown that significantly more patients who received initial combination therapy with infliximab and methotrexate were able to discontinue infliximab, compared to those with delayed introduction of the biologic agent (56% vs. 29%, $p=0.008$) [7]. In the OPTIMA trial RA patients with early RA who achieved stable low disease activity on adalimumab plus methotrexate who withdrew adalimumab mostly maintained their good responses [8]. Even in more established RA, discontinuation of adalimumab can be feasible but mainly for patients on deep remission, as shown in the HONOR study [9].

Taken together, most of the available data on discontinuation of TNF-inhibitors comes from non-randomized trials or from early RA patients enrolled in double-blind clinical trials. These results may not be applicable to many patients with long standing disease seen in clinical practice.

The aim of this pilot study was to assess the possibility of discontinuing adalimumab treatment while maintaining remission in RA patients with established disease in stable clinical remission on combination therapy with adalimumab + methotrexate (MTX).

Methods

Study design

This was a multi-center, randomized, controlled, open-label, pilot study. The main inclusion criteria were: age ≥ 18 ; diagnosis of RA based on 1987 revised American College of Rheumatology (ACR) classification criteria[10], positive rheumatoid factor (RF) or at least one erosion on the radiograph of hands or feet; treatment with adalimumab in the approved dose of 40mg every other week for at least 6 months; concomitant treatment with methotrexate (MTX) in a dose of at least 10mg per week for a minimum of 6 months (stable dose for a minimum of 2 months); stable remission according to the Disease activity score (DAS) 28[11], based on 28-joint counts, ($DAS28 < 2.6$) for at least 3 months based on assessments at baseline and on at least one more occasion 3-6 months prior to baseline, documented in patient record or registry. Concomitant corticosteroids were allowed if the dose was 10mg per day or less (prednisolone or equivalent) and has been stable for at least 3 months at study entry.

Patients fulfilling the inclusion criteria were randomized in a 1:1 ratio to arm AM (continue with ADA and MTX) or to arm M (discontinue ADA and continue with MTX monotherapy) for 52 weeks (figure 1). Any patient experiencing a 'flare' at any visit should continue in the rescue arm, where ADA would be reinstituted. Flare was defined as $DAS28 \geq 2.6$ or an increase in $DAS28$ ($\Delta DAS28$) > 1.2 from baseline at any time. After week 52 an observational extension phase ensued where patients were treated at the discretion of the investigator for an additional period of 52 to 104 weeks.

The study was conducted in accordance with the protocol, International Conference on Harmonization (ICH) guidelines, applicable regulations and guidelines governing clinical study conduct and the ethical principles that have their origin in the Declaration of Helsinki. It was registered at Clinical Trials.gov (NCT00808509).

Endpoints

The primary endpoint of this trial was the proportion of patients in $DAS28$ remission at week 28. Secondary endpoints included incidence of flare and the evolution of physical function (assessed using the Health assessment questionnaire (HAQ)[12]).

Further exploratory analyses were performed and included the following endpoints:

- 1) Incidence of flare ($DAS28 \geq 2.6$ or a $\Delta DAS28 > 1.2$ from baseline at any time.
- 2) Incidence of at least one $DAS28 \geq 2.6$ from baseline to week 28
- 3) Incidence of at least one $\Delta DAS28 > 1.2$ from baseline to week 28
- 4) Proportion of patients with at least one of the following from BL to week 28:
 - a. $\Delta DAS28 > 0.6$
 - b. $DAS28 \geq 2.6$ AND $\Delta DAS28 > 1.2$
 - c. $DAS28 \geq 2.6$ AND $\Delta DAS28 > 0.6$
- 5) Proportion of patients in $DAS28$ remission at week 52
- 6) Flare-free survival during first 28 weeks

- 7) Change in functional status (assessed by HAQ) at week 28
- 8) Change in radiological status (analysis of X-ray data at week 52)

In a secondary analysis we applied the EULAR/ACR Boolean remission criteria to the two arms and assessed the frequency of remission. Remission was defined according to these criteria as swollen joints count ≤ 1 and tender joints count ≤ 1 and patient global assessment ≤ 1 (on a 0-10 scale) and ESR (erythrocyte sedimentation rate) ≤ 20 mm/hour[13, 14].

Analysis of radiological data, safety data

X-rays of hands (PA view) and feet (AP view) were performed at baseline (unless comparable radiograph had been obtained within 3 months from baseline), at week 52 and at week 104-156. The Sharp/van der Heijde (SvH) scoring method was used to assess radiological progression[15]. Adverse events were assessed at every visit throughout the study.

Statistical analysis

Proportions were compared between the two arms using Fisher's exact test. Continuous variables were presented as median (IQR=interquartile range) and were compared by the non-parametric Mann-Whitney test. The Wilcoxon Signed Ranks test was used as non-parametric paired test. Survival curves representing patients free of flares were compared by Log-Rank test. Analyses were performed by intention to treat (ITT) with non-responders imputation for patients in both groups who experienced flare.

Results

Patient population

Patient disposition is shown in figure 2. From a total of 237 screened patients only 33 patients (14%) were enrolled in the study. A significant number of patients (30%) were not willing to stop adalimumab; 16% of patients did not fulfill the criteria for stable remission; 12% were on adalimumab monotherapy or had a lower dose of methotrexate than 10mg/week; 5% had another dose of adalimumab than 40mg every other week. The remaining 40% of patients screened could not be enrolled for other reasons. Of the 33 patients enrolled, 17 were randomized to arm AM and 16 to arm M. One patient was later excluded from each arm, one in the M arm due to a major protocol violation at week 8 and one in the AM arm who did not fulfil the inclusion criteria. Patient baseline characteristics from the time of randomization are shown in table 1.

Primary, secondary and exploratory endpoints

At week 28, 15/16 patients in AM group and 5/15 patients in M group were in remission ($p=0.001$) (figure 3-A). Two patients in the M group who did flare did not want to restart ADA. The proportion of patients with at least one flare (defined as at least one DAS28 ≥ 2.6 or

Δ DAS28 >1.2) during the first 28 weeks was 50% (8/8) in AM group and 80% (12/15) in M group ($p=0.08$) (figure 3-B). Analysis on the subgroups of flare showed significant difference in the proportion of patients with at least one Δ DAS28 >1.2 (figure 3-D) but no difference in the proportion of patients with at least one DAS28 ≥ 2.6 (figure 3-C). When different definitions of ‘flare’ were tested (exploratory analysis nr. 4 as described in methods), the difference between the AM and the M group became clearer (figure 3-E, 3-F, 3-G). Survival curves suggested higher flare-free survival over time in those randomized to ADA continuation, although the difference did not reach statistical significance ($p=0.07$, figure 4).

Around half of the patients in the two arms fulfilled the EULAR/ACR remission criteria at baseline (7/16 in arm AM and 9/15 in arm M). By the end of 28 weeks, 2/16 patients in arm AM and 1/15 patients in arm M fulfilled these criteria (no statistically significant difference).

Patients who flared had longer disease duration and started treatment with ADA later than those who did not flare in both M and AM groups during the study period (table 2). There was also a tendency for lower baseline DAS28 in the non-flared patients.

At week 52 81% of patients in arm AM (13/16) and 13% in arm M (2/15) were on remission while the rest of each arm were non responders after imputation. At the observatory visit (week 104-152) 3 of the patients originally randomized to the M arm had not restarted ADA, and only 2 of these were in remission.

Functional and radiological status

In group AM the median (IQR) HAQ was 0.13 (0-0.7) at baseline and 0.32 (0-0.7) at week 28 (Wilcoxon Signed Ranks test: $p=0.8$). In group M the mean (SD) HAQ was 0.38 (0.1-0.6) at baseline and 0.5 (0.1-0.8) at week 28 ($p=0.4$). Median Δ HAQ from baseline to week 28 was 0 (0-0) in arm AM and 0 (-0.12-0.13) at arm M ($p=0.6$). The percentage of patients with at least one clinically significant HAQ increase (0.22) during the first 28 weeks was 5/16 in AM arm and 7/15 in M arm ($p=0.4$).

The median (IQR) total Sharp score at baseline was 22.5 (11.3-52.5) for AM and 42.5 (22-95.3) for M arm. One year after randomization, the total score was 25 (13.8-51.8) and 35.5 (18.3-70.8) for arms AM and M, respectively (three patients in arm M had no radiological data at year 1). No statistically significant difference was observed from baseline to year 1 for the two arms.

A total of 9 patients entered the rescue arm during the first 28 weeks. Remission was restored in 8 of them within 12 weeks (the remaining one patient achieved remission at the final observational follow-up visit (DAS28=2.5)).

Safety data

The incidence of adverse events was similar in both treatment groups with 88.2% of the patients in the adalimumab + MTX arm and 100.0% of the patients in the MTX arm reporting

at least one adverse event. No adverse events were reported to cause discontinuation of study treatment or death. One serious adverse event (femur fracture) was reported by a patient in the adalimumab + MTX arm and three (malignant melanoma, chest pain and pleuritis) in the MTX arm.

Discussion

In this randomized, open-label, pilot study, RA patients with established disease who were in stable remission under treatment with adalimumab and methotrexate rarely maintained remission after discontinuation of the biologic agent. This result is in agreement with previous discontinuation trials, such as the ATTRACT trial [6].

Several important points need to be considered when interpreting these results. First, patients enrolled in this study had long-standing disease (median 8 years). The results should therefore be applicable to RA patients with more established disease. In early disease the possibility of discontinuing the biologic agent while maintaining remission seems to be greater, as shown in other trials[7][8].

A second important point is the definition of flare. It is obvious from figures 2A, 2B and 2C that the way to define flare greatly influences the results. When a strict definition of flare was used (a single $\text{DAS28} \geq 2.6$), then the difference between the AM and M group was no longer significant, since as many as half of patients continuing with ADA flared. This was not unexpected, as DAS28 can vary normally and can be slightly increased even in the absence of a true clinical flare. When different definitions of disease deterioration were used, for example a combination of DAS28 and ΔDAS28 with a specified minimum increase in their value, the difference between the two arms became more obvious. This criterion might be more clinically meaningful. An important lesson from this study is therefore the choice of flare definition for future studies. When the ACR/EULAR criteria were applied very few patients remained in remission and no difference between groups could be detected.

At the end of 28 weeks the majority of patients in the MTX monotherapy arm flared, but 3 patients (20%) had a sustained remission after ADA discontinuation. At the extension visit (week 104-156) 3 patients originally allocated to ADA discontinuation had not yet restarted ADA, and of these 2 were in remission.

This suggests that anti-TNF discontinuation might be feasible even in established RA, but only for a small group of patients. Identification of these patients is of course of interest. As shown in table 2, there was a tendency for lower DAS28 at the time of ADA discontinuation as well as shorter time from disease onset to ADA initiation in the subgroup of patients who did not flare, suggesting that the ‘‘depth’’ of remission according to DAS28 as well as the earlier initiation of biologic DMARD might increase the chance of retaining remission after discontinuation of the biologic agent.

Last but not least, all patients experiencing a flare who restarted treatment with ADA again achieved DAS28-defined remission without any unexpected safety signals. There was no progression of joint damage or deterioration of physical function in either group.

The study has limitations, such as the small number of patients, the open label design, and the strict definition of flare, as discussed above. A “nocebo” effect (the reverse of the placebo effect, where the patient’s expectation of getting worse causes an actual worsening) might partly contribute to the higher flare rate in the ADA discontinuation group. On the other hand there are important strengths, such as the randomized, controlled character of the trial, the homogeneous population of patients and the relevance to the payer perspective on clinical practice.

Conclusions

In this pilot study, remission was rarely maintained in patients who discontinued adalimumab. Compared with patients who continued combination therapy, the proportion of patients with sustained remission in the discontinuation group was significantly lower for the primary endpoint and most secondary endpoints. However, adalimumab discontinuation may be feasible in a minority of patients with established RA in stable clinical remission on adalimumab plus methotrexate.

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Tables and figures

	AM Group (N=16)	M Group (N=15)	All (N=31)
Age [years, median (IQR)]	56 (38.8–62)	64 (59–66)	61 (53–65)
Sex (female)	10/16 (62.5%)	10/15 (66.7%)	20/31 (64.5%)
Disease duration [years, median (IQR)]	7.6 (4.0–12.1)	10.4 (5.2–19.2)	8.0 (4.8–16.2)
Time (months) on adalimumab at baseline [median (IQR)]	26.5 (12.5–51.2)	43.3 (11.7–51.5)	29.1 (12.3–51.2)
Time from RA diagnosis to adalimumab start [years, median (IQR)]	3.2 (2.2–9.5)	6.5 (4.1–15.1)	4.8 (2.8–10.4)
RF (positive)	11/16 (68.8%)	11/12 (91.7%)	22/28 (78.6%)
Anti-CCP (positive)	5/10 (50%)	8/9 (88.9%)	13/19 (68.4%)
No. of previous DMARDs [median (IQR)]	2 (1–3)	2 (2–3)	2 (1–3)
No. of previous biologics [median (IQR)]	0 (0–1)	0 (0–1)	0 (0–1)
Baseline DAS28 [median (IQR)]	2.13 (1.6– 2.4)	1.69 (1.5– 2.37)	1.9 (1.55– 2.39)
Baseline HAQ [median (IQR)]	0.13 (0–0.72)	0.38 (0.13– 0.63)	0.38 (0– 0.63)
Concomitant MTX dose [median (IQR)]	20 (15–20)	20 (10–20)	20 (15–20)

Table 1. Baseline characteristics in the two treatment arms.

IQR: interquartile range; RF: rheumatoid factor; anti-CCP: anti-cyclic citrullinated peptide antibodies; DMARD: disease modifying antirheumatic drugs; DAS28: disease activity score based on 28 joints; HAQ: health assessment questionnaire

	AM Group	AM Group	M Group	M group	All patients	
	Flare (N=8)	No flare (N=8)	Flare (N=12)	No flare (N=3)	Flare (N=20)	No flare (N=11)
Age [years, median (IQR)]	51.5 (35-60.5)	59.5 (53-62.8)	63.5 (59.5-66)	65 (33-65)	61.5 (53.8-65.5)	61 (52-65)
Disease Duration [years, median (IQR)]	11.2 (7.0-22.3)	4.8 (3.0-8.2)	10.9 (5.2-21.9)	5.5 (4.8-14.6)	10.9 (5.7-21.9)	5.2 (3.7-8.2)
Time from RA diagnosis to ADA [years, median (IQR)]	8.5 (3.4-20.7)	2.5 (0.5-3.3)	7.6 (4.1-16.2)	4.8 (3.8-10.3)	8.5 (4.1-16.3)	3 (1-4.8)
Time on ADA treatment [years, median (IQR)]	2.0 (1.2-4.1)	2.4 (0.7-4.7)	3.8 (1.5-4.3)	1.0 (0.7-1.0)	3.0 (1.4-4.2)	2.3 (0.7-4.3)
DAS28	2.4 (2.1-2.5)	1.8 (1.3-2.3)	1.7 (1.5-2.5)	1.7 (0.5-1.8)	2.3 (1.7-2.5)	1.7 (1.3-1.9)
SJC	0 (0-1.75)	0 (0-0)	0 (0-1)	0 (0-0)	0 (0-1)	0 (0-0)
TJC	0.5 (0-2)	0.5 (0-1.8)	0 (0-1)	0 (0-0)	0 (0-1.8)	0 (0-1)
ESR (mmHg)	9 (5.8-15.5)	6 (4.5-10.5)	8 (5-10)	10 (2-10)	8 (5-11)	6 (4-10)
CRP (mg/L)	3 (1.1-5.0)	4.5 (0.6-7.0)	4.5 (2.5-6.6)	2.9 (1.4-5)	4 (1.6-5.2)	2.9 (1-7)
GH	10 (8-20.5)	6 (1.5-7.8)	5.5 (0.5-10)	5 (1-13)	8 (4.3-15.3)	6 (1-8)
HAQ	0.3 (0-0.9)	0.13 (0-0.7)	0.4 (0.1-0.7)	0.4 (0.3-0.5)	0.4 (0-0.7)	0.3 (0-0.5)
Methotrexate dose (mg)	20 (15-20)	20 (15-20)	18.75 (10.6-20)	20 (10-20)	20 (13.1-20)	20 (15-20)

Table 2. Baseline disease characteristics for patients who flared and those who did not flare during the first 28 weeks (totally and in the two treatment arms). Values in bold differ significantly between flare and no flare groups ($p<0.05$).

IQR: interquartile range; DAS28: disease activity score based on 28 joints; SJC; swollen joint count; TJC: tender joint count; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; GH: global health assessment (patient's visual analogue scale); HAQ: health assessment questionnaire;

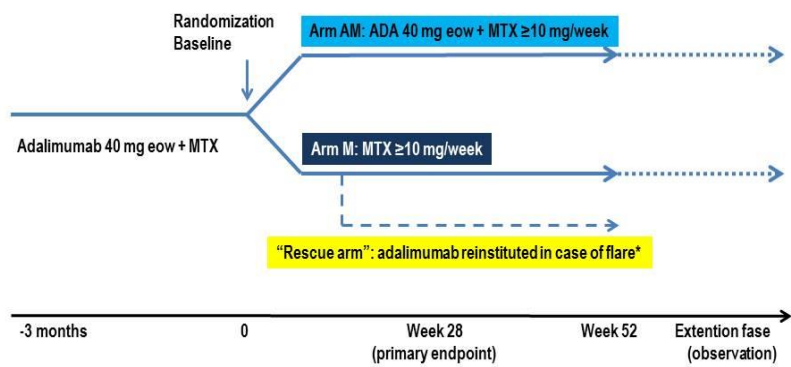


Figure 1. Study design

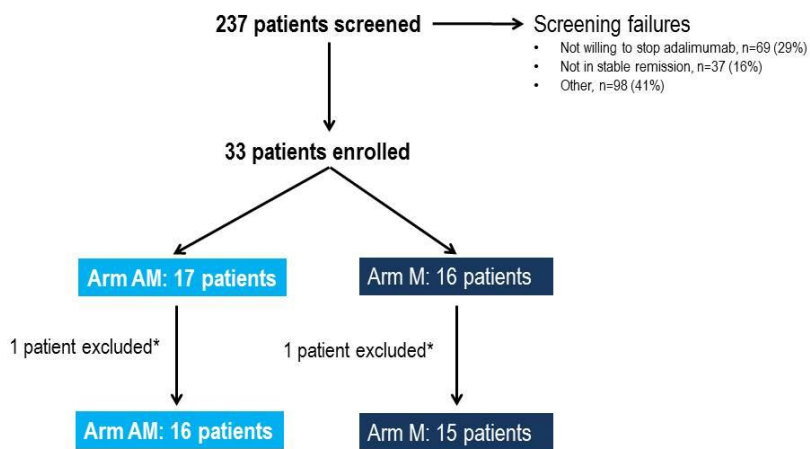
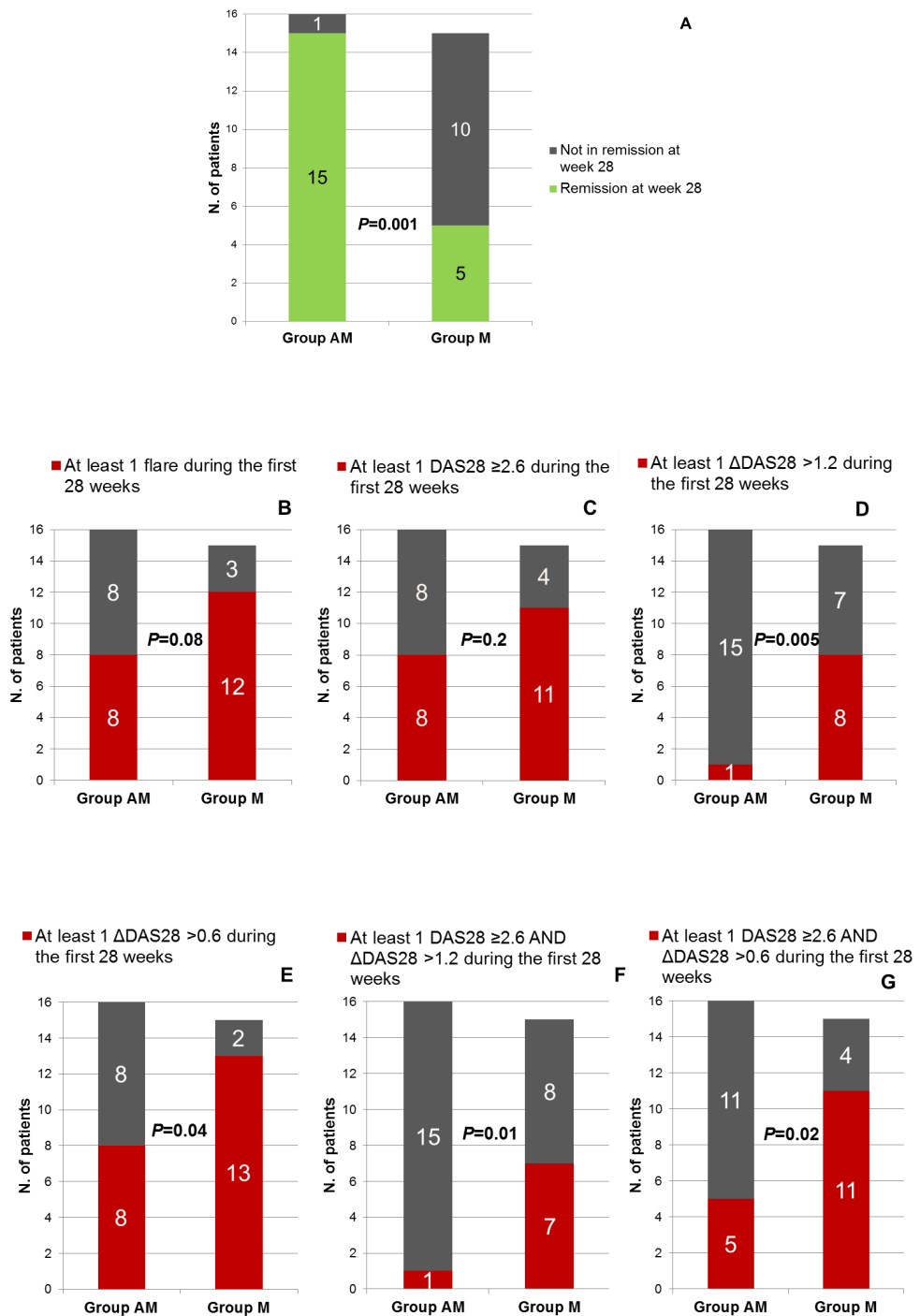


Figure 2. Patients' disposition.

Figure 3. Primary (A) and secondary (B-G) endpoints.



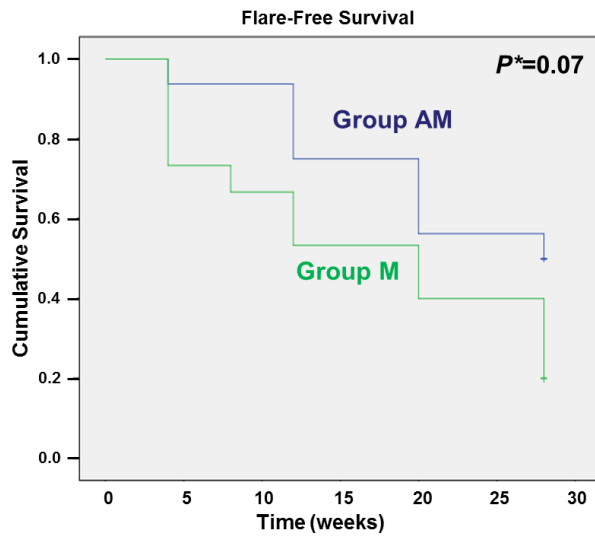


Figure 4. Flare-free survival.

